SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL EVALUATION OF NANO-PARTICLES PREPARED BY USING THE EXTRACTS OF Viola pilosa AND Skimmia laureola

BY

MARIA KHAN PANNI

A dissertation submitted to The University of Agriculture Peshawar in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY IN BIOTECHNOLOGY AND GENETIC ENGINEERING

INSTITUTE OF BIOTECHNOLOGY AND GENETIC ENGINEERING

FACULTY OF CROP PRODUCTION SCIENCES

THE UNIVERSITY OF AGRICULTURE

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JANUARY, 2018
DEDICATION

To my Ammi Jee
&
to my Papa Jani & Mama
This humble work is a sign of
My love to you!

Maria Khan Panni
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MARIA KHAN PANNI
SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL EVALUATION OF NANO-PARTICLES PREPARED BY USING THE EXTRACTS OF Viola pilosa AND Skimmia laureola

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ABSTRACT

Multiple antibiotic resistant strains of microorganisms are imposing a dire necessity for novel and consistent drugs. Reliable natural products with negligible side effects are required to resist these pathogenic assailants. Investigation of medicinal plants for pharmacological and qualitative properties is substantial for appropriate choice and treatment of various diseases. The current study was aimed to Biosynthesize and characterize nanoparticles of boiled extract of plants and to evaluate different extracts of the plants qualitatively. The gold and silver nanoparticles (AuNPs and AgNPs) were successfully synthesized from solvent extracted methanolic crude and aqueous fractions (50mg plant extract / 100 ml de-ionized H₂O) of the test plants under study. A solution of .1 mM of AuCl₃ was shaken with Viola crude, aqueous and Skimmia stem aqueous extracts in 4:1 while 3:1, 2:1 and 1:1 in other samples for the production of gold (Ag) nanoparticles (NPs). For the synthesis of AgNPs from test plants, a solution of .1 mM of AgNO₃ was combined with Viola shoot crude, aqueous and Skimmia leaves crude extracts in 10:1 (10 ml of AgNO₃ and 1ml extract). Similarly, 1:5 (1ml of AgNO₃ and 5ml extract) with Viola root crude, aqueous and Skimmia stem and leaves aqueous extracts and 1:10 stem crude extracts. Visual observation in color change from yellowish to dusky purple and dark brownish was taken as initial formation of silver and gold nanoparticles respectively. The formation of NPs synthesis was reaffirmed by the existence of observed peaks in the optimum range of 350-450 nm (silver Nps) and 500-600 nm (gold Nps) through UV-Vis spectrophotometer. The gold and silver nanoparticles of Viola were found highly stable at the temperature range between 25°C and 50°C and that of Skimmia between 20°C and 40°C and more stable at salt stress in milli-Molar concentrations as compared to molar ones. The X-Ray diffraction showed crystalline, spherical structure, and centro symmetric and cubic nature of gold and silver nanoparticles. The results of SEM confirmed the size of AuNPs between the range of 20 nm – 50 nm and that of AgNPs in 20 nm – 80 nm range. The FT-IR studies showed that phenols and Esters were accountable for the green production of the Gold and Silver nanoparticles (AuNPs and AgNPs). The silver nanoparticles (AgNPs) were found more active in regulating the development of bacterial and fungal strains as compared to gold nanoparticles (AuNPs). More significant results were recorded for roots of Viola pilosa (90.25%) and leaves of Skimmia laureola (86.79%) against bacterial strains. Similarly, shoots extracts of Viola pilosa (95.02%) and stem extracts of Skimmia laureola (97.29%) were found more efficient in antifungal activities. The antioxidant potential of nanoparticles proved that they were active in scavenging free radicals possessing maximum antioxidant potential of 80.86% when compared to the
control. Similarly, roots extracts of *Viola pilosa* were by far most potent in scavenging free radicals amongst all the other solvent extracts and showed much significant antioxidant activities of 95.87% at 250 µg/ml. The plants also presented significant level of phytotoxic (97.76%) and insecticidal activities (30%). Phytochemical study of the test plants discovered that they were abundant in Tannins, Carbohydrates, Sterols, Proteins and Lipids. Based on these results, both the tested plants are highly recommended for Green synthesis of nanoparticles as well as efficient Antibacterial, Antifungal and Antioxidant agents.

**Keywords:** *Viola pilosa, Skimmia laureola*, antibacterial, antifungal, antioxidant, nanoparticles, insecticidal.
I. INTRODUCTION

Plant containing active chemical components used for the management of infectious diseases in humans and wildlife is categorized as medicinal plant (Redzic, 2007). Since primitive times, the manhood has used therapeutic plant life in an effort to cure infections and get rid of corporal pain. Plants are renowned to produce certain compounds which are toxic to microbes. An improved interest has arisen during the last two decades to explore the bioactive compounds of intrinsic and adapted plant species for medication and dietetic practices (Ho et al., 1992; Oktay et al., 2003; Wangensteen et al., 2004) through the documentation that phytochemicals do have pronounced future as sources of pain relievers (Cragg et al., 1996). Phytochemicals are rich in natural and active compounds that are full of antimicrobial properties (Bakht et al., 2011 a, b, c and d; 2012; 2013 a, b; 2014 a, b, c; Ali et al., 2015).

Medicinal plant extracts and phytochemicals nowadays are receiving significant position as a credible source for hindering the growth of various pathogens in the modern era.

Indigenous specifics are as ancient as human civilization itself, however the term ethno botany was conveyed by an American botanist named John Hershberger (1896) to discover novel medicinal plant life utilized by native societies. Ever then it was well-defined as the indigenous understanding of traditional people, regarding nearby plants diversity and as an information that exactly how, the individuals of specific culture and area make the use of these native plant species (Kumar et al., 2014). Plant life having therapeutic activities hold diverse bioactive natural compounds that are helpful for the treatment of various animal and human infections. To encourage the accurate use of herbal treatment and to find out its possible strength as foundation for novel medicines, it is indispensable to explore therapeutic plants with an endless status in a much strengthened way (Schopen, 1983; El-Faky et al., 1995; Awadh et al., 2001).

Various pathological conditions in human being that are really hard to treat through traditional medicine are frequent (Redzic, 2007). From the most primitive ages manhood has been using therapeutic plant life in an effort to cure infections and relieve physical pain (Hamayun et al., 2006). Mother Nature has always helped as a
gorgeous source of healing for centuries and a remarkable amount of existing medicine has been taken through traditional materials particularly from plant sources (Cowan, 1999). One of the basic essentials for the achievement of community health is the accessibility and proper usage of appropriate medicines. Plant life has at all times been there as an important source of treatment, either as old-fashioned mixtures or in the form of pure bioactive ingredients (Farnsworth et al., 1985). From the fossil record analysis of humans, it is shown that they have always used medicinal plant life as treatment, about 60,000 years back (Solecki and Shanider 1975).

Nowadays, a larger population of the whole world is converging towards traditional medicines extracted purely from plant sources and the reason is the adversative side effects of the modern drugs (Rios and Recio 2005). Appearance of various stubborn species of microbes due to massive usage of antibiotics against contagious infections has resulted in an improved importance of traditional medicines (Chopra et al., 1997). According to an estimate, approximately 20,000 plant types are being used as therapeutic medicines throughout the globe. Worldwide, nearly 70% of the traditional practitioners, rely only on plants as therapeutic sources for curing various types of diseases. In sub-continent, plant based drugs have been found to be extensively used from a long period of time. According to a survey, local experts commonly known as Hakeems in Pakistan are involved in the treatment of approximately 60% of the general populace. Moreover, the analysis revealed that these traditional experts are treating 60% population in Indonesia, 65% in Srilanka, 75% in Nepal, 80% in India, 85% in Myanmar, and 90% in Bangladesh. In China around 40% of the overall medicinal products are used in traditional medical practices (WHO, 2002).

In the past few years, a thorough investigation is being endured for constituents with significant antibacterial properties. Plants are notorious for producing certain compounds which serve as a natural toxin to pathogens. In bryophytes, the material barriers are least effective and, as a consequence, the synthesis of specific molecules, phenolic compounds with antimicrobial property: the so called chemical barriers (Harborne, 1988), is the effective most defense mechanism. Defense related substances fit into a wide range of chemical categories
including flavonoids and iso-flavonoids. Bi-flavonoids in mosses are also labelled as potential chemical barriers against different micro-organisms.

The epidemiology records show that one of the main causes of public health issues is the prevalence and dominance of different mycoses. Due to the frequent use of antifungal agents, the microbes are getting resistant to these drugs day by day. An improvement in the drug-resistant fungal strains along with the reduced amount of available drugs make it quite essential to find out new antifungal agents from natural sources primarily medicinal plants. (Aqil et al., 2010). Antifungal therapy has been playing a major part in health and care practices. Medicinal plants are a vital cause of unique antifungals. (Webster et al., 2008).

Studies revealed that consumption of antioxidants like vitamin C decreases the likelihood of cardiac diseases and cancer (Marchioli et al., 2001). Risks of cardiac arrest can be reduced which is possible either by strengthening the body's own regularity mechanism or by use of recognized dietetic oxidant containing supplements. (Stanner et al., 2004). Numerous studies discovered that the phenols, primarily flavonoids, from particular medicinal plants are innocuous and possess antioxidant activities that could be used against antiviral, anti-inflammatory, antitumor, anti-carcinogenic, antibacterial and anti-mutagenic special effects (Ozgova et al., 2003).

In plants different derivatives of phenylpropanoid, shikimate, pentose and phosphate pathways are found that are basically the phenolic compounds or simply the secondary metabolites (Randhir et al., 2004). Such type of secondary metabolites, as one of the broadly occurring class of phytochemicals, hold a substantial biological as well as morphological significance in various plant species. (Bravo, 1998). The unique beneficial antioxidant properties of phenolic compounds are considered as the most valuable derivative effect (Heim et al., 2002). Phenolic compounds may perhaps be the most important element of radical scavenging activity of various food items (Parr and Bolwell, 2000), and may possibly contribute as a natural base for various antioxidants. For this reason, in modern era, significant attention is being focused towards identifications of plant species with antioxidant potential that might be a source for human expenditures.
Medicinal plants are rich in compounds which serve as natural insecticides. Insecticidal activities of numerous plants against different insects have been carried out (Umadevi et al., 2013). The therapeutic property of ethno botanical florae is mainly due to the presence of phenolic compounds that are responsible for providing protection against pathogens like microbes and insects by affecting numerous characteristics of pests’ larvae, such as growth, development and survival rate (Maerz, 2005). The deadliness of secondary metabolites has been measured by spotting increase in mortality rate of larvae (Blaney et al., 1984; Chew, 1985; Krischik et al., 1991; Poykko and Hyvarinen 2003; Dyer et al., 2003; Ghosh et al., 2008).

Nanotechnology is nowadays creating an increasing sense of interest in science particularly biotechnology and different biomedical techniques (Prabhu et al., 2010). Nanoparticles show entirely new and enhanced potential built on definite features such as morphology, size and scattering. The nanoparticles have countless and significant uses. Traditionally, silver has been notorious for its disinfecting influence and has been used in various functions extending from old-fashioned drugs to gastronomic objects. It is also stated that silver nanoparticles (AgNPs) are innocuous to human beings and are really efficient against fungi, bacteria, viruses and many other eukaryotic organisms at even small dosages with out any bad after effects (Jeong et al., 2005).

A number of microbes are getting resistant against antibiotics used in the modern healing practices. To get rid of this problem, nano-particles are synthesized and used effectively as drug carriers (Raveendran et al., 2003). Moreover, a number of silver salts and their respective byproducts are synthesized as commercial antimicrobial agents (Krutyakov et al., 2008). Silver when used in a slight concentration is innocuous to humans, but are really fatal to microbes (Sharma et al., 2009). Antimicrobial activities of silver nanoparticles (AgNPs) permit them to be suitably used for several domestic purposes such as yard goods, foodstuff storing bottles, household machines and in medicinal techniques (Marambio-Jones and Hoek, 2009). The supreme use of silver nanoparticles is in therapeutic production like tropical liniments to avoid contagion against open wounds and burns (Ip et al., 2006).
The expansion of consistent technology to create nanoparticles is an essential feature of nanotechnology (Natarajan et al., 2009). Nanotechnology basically is an enabling tool that works with nano-meter sized substances (Feyman, 1991). Different methods are used to synthesize nanoparticles, which includes physical, biological and chemical. The biological method (Green synthesis) of biocompatible, clean, innocuous and environmentally benign nanoparticles, both extra-cellularly and intra-cellularly is preferred (Raveendran et al., 2003). Green nano-biotechnology is the creation of nanostructures by means of living entities. Recently living cells were used to synthesize nanoparticles. Silver nanoparticles were synthesized extra-cellularly by using the fungus *Aspergillus fumigatus* (Mann, 1996). Different bacterial and fungal strains can also be employed to synthesize gold and nanoparticles (Phillip et al., 2006). Amongst living entities, plant life has profound applications mostly in synthesis of metal nanoparticles. Biosynthetic practices of nanoparticles would be much more effective if nanoparticles are created extra-cellularly by plants extracts and in an organized mode according to their shape, size and dispersity. Large scale production of nanoparticles can also be carried out by appropriate use of plants (Kumar and Yadav, 2009).

Natural synthesis of nanoparticles by medicinal plants is currently under study for its antimicrobial properties (Calvo et al., 2001; Bhyan et al., 2007; Khandelwal et al., 2010; Saxena et al., 2010; Thirumurgan et al., 2010). For the past few years, substantial work has been carried out to synthesize novel medicines from natural sources due to the opposition of biological entities to the current medicines. Mother Nature has always been a vital cause of the biological goods presently being used in therapeutic practices (Thirumurguan et al., 2009).

Characterization of phyto nanoparticles is essential for better understanding, proper control and wide scale applications. There is a range of practices used for the characterization of different nanoparticles including atomic force microscopy or AFM, ultraviolet-visible spectroscopy (UV), nuclear magnetic resonance (NMR), Scanning electron micrographic analysis (SEM), Fourier transform infrared spectroscopy (FT-IR), Transmission electron microscopy (TEM) and X-ray diffraction (XRD). All these techniques are usually light-based. However, now a days, in order to measure the instant concentration, size and surface charge of a diverse range of nanoparticles, a non-optical practice named as Tunable Resistive Pulse
Sensing or simply (TR-PS) is being devised for characterization purposes (Prime and Whitesides, 1991).

*Viola pilosa* or *Viola serpens* belongs to family Violaceae (frequently known as Leoniaceae, Retrosepalaceae or Alsodeiace) which include about twenty genres and around 800 species (Mabberley, 1987). *Viola* is a member of the genus of flowering plants in the violet family. It is the major genus in the voilet family covering about 525 to 600 species. In Pakistan, it is characterized by genus (*Viola*) and 17 different species (Perveen and Qaiser, 2009). *Viola pilosa* or *Viola serpens* Wall, is generally known as smooth leaf white violet or “Banafsha”. It is commonly found in soggy woods and mountainous areas of Himalayas, from Afghanistan to South West China, South East Asia and Burma at heights of about 1200-3000 m. Flowering of *Viola pilosa* usually take place in March-May. Typically, *Viola pilosa* is found in Swat and Siran valleys of Pakistan. (Qaiser and Omer, 1985).

*Viola pilosa* has been used to treat innumerable diseases. The whole plant of *Viola serpens* wall is used as decoction orally as a cure for hepatitis and jaundice (Abbasi et al., 2009). It has also been reported that the flowers and leaves of *Viola serpens* are purgative, astringent, refrigerant, demulcent, emollient, diuretic and diaphoretic (Haq and Rehman, 1990; Shinwari and Khan., 2000; Ahmed et al., 2006; Muhammad et al., 2012c). Isolation of compounds from *Viola serpens* revealed that it has an ample amount of glycosides and flavanoids (Adhikary et al., 2011).

*Skimmia laureola* or *S. laureola* typically entitled as Nazar Panra is a member of family Rutaceae. In Pakistan, it is commonly found in region of Hazara, Murree, Kashmir valley, Upper Swat and Shangla. Healing properties of the *S. laureola* have been acknowledged in different cultures globally. It has been reported as a remedy against headache, fever and cold (Hamayun, 2007). Dry leaves smoke has been used as a treatment for nasal tract blockage. Moreover, *Skimmia laureola* leaf has also been commended for several other problems like cough relieve and as insecticide and pesticide (Qureshi et al., 2009). Leaves harvested from plant are commercially used in various foodstuff as additives, in old-fashioned therapeutic and traditional medicines and are really considered as sacred (Bhattarai et al., 2009). A complex anthelmintic property was observed in the essential oils extracted from the plant (Mehmood et al., 2011).
Objectives of the study:

1. To biosynthesize and characterize nanoparticles of boiled extract of *Viola pilosa* and *Skimmia laureola*.
2. To investigate anti-bacterial, anti-fungal and anti-oxidant activities of Nanoparticles of different extracts of *Viola pilosa* and *Skimmia laureola*.
3. To investigate anti-bacterial, anti-fungal and anti-oxidant activities of different extracts of *Viola pilosa* and *Skimmia laureola*.
4. To evaluate different extracts of *Viola pilosa* and *Skimmia laureola* qualitatively.
Naraginti and Li. (2017) developed a one-step quick and cost friendly green synthetic technique using the fruit extracts of *Actinidia deliciosa* for the creation of much stable and multi-functional gold and silver nanoparticles. These nanoparticles were then studied for the biological properties based on extremely stable antibacterial and antioxidant effects. The results from Transmission Electron Microscopy exposed the spherical shape of silver NPs having diameters ranging between 25 to 40 nm, while the AuNPs showed a size of particles ranging between 7 and 20 nm. X-ray diffraction analysis of gold and silver nanoparticles revealed the face-centered like cubic structure. The FTIR (Fourier-transform infrared) pattern specified the occurrence of promising functional groups responsible for nanoparticles capping. The *P. aeruginosa* specie was used for inspecting the antimicrobial potential of the silver and gold nanoparticles in which TEM spectra showed the damaging of the cell membrane.

Almalki. (2017) investigated the antifungal, antibacterial and antioxidant properties of the crude seed extracted samples of *Ruta chalepensis, Citrullus colocynthis, Salvia aegyptiaca, Hyoscyamus muticus, Amaranthus lividus* and *Ocimum basilicum*. The broth dilution technique was used for the antifungal and antibacterial activities. The interacellular protein leakage, quantification of alkaline phosphatase and lactate dehydrogenase enzymes were used for testing the effects of the crude extracts on the microbes. The antioxidant activity was carried out by using DPPH radical scavenging assay, hydroxyl radical scavenging assay, reducing power and superoxide radical scavenging assays. The outcomes showed the maximum activity of *C. colocynthis* against the microorganisms under study, with the MIC values ranging between 100-150 μg/ml (Gram positive bacteria) and 100 - 250 μg/ml (Gram negative bacteria). While the MIC for *R. chalepensis*, *O. basilicum* and *H. muticus* against tested fungal strains ranged between 100 - 500 μg/ml respectively. The maximum activity of ALP was showed by *K. pneumonia* with extract when compared with control, while the *S. aureus* and *E. coli* strains gave higher concentrations of LDH and proteins. The study concluded that the seed extracts exhibit significant antimicrobial as well as antioxidant propereties. Hence these
medicinal plants can be an outstanding addition to the natural antioxidants and antimicrobial agents for medicinal purposes.

Ahmed et al. (2015) selected water extracted leaves samples of *Skimmia laureola* for the production of silver nanoparticles at room temperature. The variation in color of reaction mixture from yellow to dark brown was observed during the process of silver NPs synthesis. The XRD and SEM techniques were used for the confirmation of spherical, hexagonal shapes and crystalline nature of the nanoparticles. The bioactive compounds of leaves extracts such as coumarins, tritepenoids and skimmidiol were essentially involved in the creation of nanoparticles. Moreover, the aqueous leaf extracts along with their silver nanoparticles were seen with remarkable antimicrobial activities against human pathogens under study and were suggested as appropriate nanomaterial for further biomedical applications.

Zeb et al. (2015) evaluated the antibacterial activities of cold and hot water extracts of *Skimmia laureola* against four different strains of bacteria including *Proteus mirabilis, Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis*. Nutrient agar well diffusion technique was used for evaluating the antimicrobial nature of the plant. The outcomes revealed that the cold and hot water extracts of *Skimmia laureola* showed moderate antibacterial activities against all the tested micro-organisms.

Razzaq et al. (2015) carried out research on the medicinal plant life at higher altitude of District Shangla, Khyber Pakhtunkhwa, Pakistan. The research process discovered 25 therapeutic plant species fitting into 21 diverse families. Amongst them, 19 were titled as herbs, 3 as shrubs, 2 classes were climbers and one was found to be tree. The most significant species were: *Geranium wallichianum, Valeriana jatamansii Berberis vulgaris, Aconitum violaceum, Paeonia emodi, Aconitum heterophyllum, Paeonia emodi, Viola canescens* and *Podopyllum emodi* etc. They concluded that the medicinal plant biodiversity of the region is going through high biotic pressure because of uncritical deforestation, over grazing, habitat demolition, population growth, unscientific assortment and creation of hostile species. Therefore, it is the dire need of the day to conserve and protect the medicinal plant biodiversity of the region.
Dastagir and Hussain. (2013) studied the phytotoxic potential and insecticidal activities of members of family *Euphorbiaceae* and *Zygophyllaceae*. The statistical analysis showed the significant growth inhibition of *Lemna minor* by the extracts (10g and 20g) of all the tested plants under study. The total means of plants presented non-significant values whereas a significant interaction was found between plants and their extracts. They also concluded that significant inhibition in growth of *Lemna minor* was also caused by different dilutions of *Chrozophora tinctoria*, *Ricinus communis*, *Fagonia cretica*, *Tribulus terrestris* and *Peganum harmala*. There was an insignificant interaction amongst dilutions and plants. Amongst the three tested plants, the maximum insects mortality rate was showed by *Peganum harmala* followed by *Fagonia cretica* at the equivalent dose. The lower mortality rate for *Tribolium castaneum* was showed by *Tribulus terrestris*. The study concluded that a significant degree of mortality of *T. castaneum* in comparison to control was presented by all the dosages (5% - 20%) of the plant extracted samples. The maximum mean of 12.8% was given by *P. harmala*, whereas the *T. terrestris* showed the lowermost dose mean value.

Barkatullah et al. (2013) evaluated the possible analgesic as well as antipyretic activity of *Skimmia laureola* by the help of ethanolic crude extracts of leaves. Various classes of phyto-constituents were determined quantitatively. The existence of sterols (81.30 ± 0.61 mg/g), alkaloids (12.50 ± 0.09 mg/g), tannins (26.83 ± 0.12 mg/g), saponins (20.93 ± 0.06 mg/g), flavoniods (12.58 ± 0.66 mg/g) and phenolic compounds (10.33 ± 0.66 mg/g) were found in leaves extracts. The result of the study supported the analgesic and antipyretic folktales of *Skimmia laureola* plant. The leaves of *Skimmia* were found to be rich in flavonoids, alkaloids, phenolic compounds, sereoles, tannins and saponins.

Muhammad et al. (2012) clarified the Ethno medicinal, pharmacological and phytochemical profile of genus Viola. They illustrated that the Viola (genus) contains approximately 500 different species broadly distributed all over the world. In Pakistan, seventeen (17) different species of viola are abundantly found. Various types of the genus have been found rich in various phytochemicals like alkaloids, caffeic acid derivatives cyclotide, flavonoids, triterpenoids and salicylic acid.
results revealed that the pharmacological and phytochemical analysis through traditional knowledge possibly will offer new and effective therapeutic pathways.

Kouvaris et al. (2012) synthesized the silver nano-particles from aqueous AgNO$_3$ by a very simpler and sustainable technique using leaves broth of Arbutus unedo (strawberry tree) that served as both reductant and stabilizer. The exposure of aqueous Ag ions to that of leaves broth caused reduction and stabilization over a period of long time, thus causing the green synthesis of surface functional AgNPs. The biologically synthesized nanoparticles were then subjected to characterization which revealed the synthesis of crystalline shaped AgNPs showing a narrow size distribution. These discrete AgNPs were coated with the leaf extracts to form smaller aggregates, to make them stable and appropriate for a long time for coatings purposes in different biotechnology techniques.

Shah et al. (2012) analyzed Skimmia laureola hydrodistillate by gas chromatography combined with mass spectrometry. The results discovered the existence of 20 different components, signifying 94.6% of the total oil. The essential oil rich in monoterpene (93.4%) was assessed for antibacterial as well as antifungal potential against seven different microbial strains by using microdilution and agar diffusion techniques. An appreciable amount of antimicrobial properties were shown by the oil against all the tested Gram-positive bacterial species, including Staphylococcus epidermidis and methicillin-resistant Staphylococcus aureus respectively. The essential oil also showed strong anti fungal activities against Penicillium chrysogenum and Aspergillus niger. They concluded that this oil can be a good addition in the preparation of antimicrobial agents.

Rehmanullah et al. (2012) studied the insecticidal, phytotoxic and cytotoxic potential using methanolic extracts of Calendula arvensis against Lemna minor, Artimiasalina (Brine shrimps) larvae and specific grain pests. The results revealed that dose dependent C. arvensis proved to be highly toxic against Lemna minor, lesser toxic at levels of 10μg/ml, 100μg/ml and a satisfactory activity at 1000μg/ml. It was also reported that a moderate level of cytotoxic activity was also found at LD$_{50}$ value 9.23μg/ml for brine shrimp larvae. The dose dependency was the major factor for insecticidal activity, whereas for similar treatments, a variable amount of vulnerability was shown by different types of insects. C. analis was found to be the most
susceptible pest with LD$_{50}$ value 0.51mg/ml, whereas, *T. granarium* was declared as the most resistant one with comparison to all the five insect types with LD$_{50}$ value of 90.50mg/ml.

Linga and Savithramma. (2011) revealed that the biologically produced nanoparticles have always been extensively applied in the field of medication. The existing research discovered the formation of silver nanoparticles from 1mM AgNO$_3$ solution by using the leaves extracts of *Svensonia hyderabadensis* as capping along with reducing agent. The nanoparticles characterization was carried out through XRD, UV-Vis Spectrophotometry and SEM techniques. The SEM and XRD analysis revealed a spherical nature with a particle size of 45nm. Moreover, these nanoparticles were also seen susceptible against various microorganisms. The study concluded that the biological production of silver nanoparticles through dried up leaves powder of *Svensonia hyderabadensis* is a conventional, cost friendly and ecofriendly process.

Methanolic plant crude extracts from medicinal plants that included *A. caudatus*, *D. gangeticum*, *S. nigrum*, *E. alba*, *P. longum* and *O. sanctum* by were used by Veeru et al. (2009) to study the antioxidant potential by means of (DPPH) free radical scavenging antioxidant assay. The ascorbic acid was taken as a standard antioxidant. The overall outcomes indicated that the strongest antioxidant activity was shown by *D. gangeticum*, followed by *A. caudatus*, *S. nigrum*, *E. alba*, *P. longum*, and *O. sanctum* respectively.

Fatimi et al. (2007) studied thirty different therapeutic plants used in Yemeni traditional medicines to treat communal infections. For this purpose 90 crude extracts using methanol, dichloromethane and aqueous were prepared. The plants were screened for antimicrobial potential against 2 Gram-negative and 3 Gram-positive bacterial species, *Candida maltose* and 5 other stubborn human fungal strains (2 yeasts, 3 hyphomycetes). Nearly all the plants revealed good antibacterial potential. Flower extracts of *Tamarin dusindica* and fruit extracts of *Ficusvasta* were found to be highly active. Out of these 30 tested plants, 13 presented about 40% antifungal potential against 1 or more human fungal pathogens. The maximum inhibition was showed by fruit of *Azimatetra cantha*, *Sansevieriae hrenbergii* and *Solanum incanum*. Ten methanolic crude extracts, specifically fruit of *Solanum nigrum* and barks of
Acacia asak barks exhibited very effective antioxidant activities in the DPPH radical scavenging assay.

Celiktas et al. (2007) used methanolic plant extracts and essential oils to investigate the antimicrobial activities of *R. officinalis* which was collected with four different time intervals of the year, from three diverse regions. The microorganisms selected for the study were *C. albicans*, *S. aureus*, *B. subtilis*, *P. vulgaris*, *S. epidermidis*, *P. aeruginosa*, *E. coli*, *K. pneumonia* and *E. feacalis*. The disc diffusion and MIC techniques were chosen to investigate the antimicrobial potential of the plant extracts and essential oils. The results indicated that the microbes under study were found completely susceptible to essential oils and moderately susceptible to the methanolic plant extracts. There was a difference in the antimicrobial properties of the oils against the microorganisms under study which was likely due to the difference in location and seasonal variations.

Chandran et al. (2006) carried out green synthesis of gold and silver nanoparticles by using a very simple technique where *Aloe vera* leaves extract was used as the reducing agent. The characterization of gold nanotriangle synthesis was done by transmission electron microscopy (TEM) and UV – vis – NIR absorption spectroscopy. The TEM technique was chosen to study the effect of concentration of reducing agent to the final yield of the reaction mixture, as well as to measure the typical size of the synthesized nanoparticles. Observing the synthesis of gold nanotriangles using TEM, it was revealed that multiple twinned particles (MTPs) played the key role in the nanotriangles formation. It was also witnessed that the particular crystalline structure of the gold nanotriangles was the result of slower rate of reaction and the shape directing outcomes of the components of the plant extracts. However, the reduction of silver ions by *Aloe vera* extracts resulted in the synthesis of spherical shaped silver nanoparticles of 15.2 nm ± 4.2 nm sizes.

Bhainsa et al. (2006) stated that the expansion of a viable and ecologically pleasant method for formation of metal nanoparticles is a substantial phase towards the applications of nanotechnology. In their work, they investigated the *Aspergillus fumigatus* for extracellular production of silver nanoparticles. The nanoparticles production method was pretty fast and nanoparticles were produced as soon as silver ions came in contact to the cell filtrate. The highest peak of 420nm, through UV–
visible spectroscopy was noticed for the aqueous medium holding silver ions. The results of TEM showed synthesis of well-defined silver nanoparticles ranging between average sizes of 5 – 25 nm. X-ray diffraction analysis of the nanoparticles showed 2θ values conforming the silver nanocrystals. They concluded that the method of extracellular reduction might result in the growth of an effective procedure for separation of nanoparticles.

Eleven different Algerian species of medicinal plants were used by Djeridane et al. (2006) in order to study the antioxidant activities and total flavonoids and phenolic content towards free radical propagation. About 70% of ethanol was used for the extraction of polyphenolics. The results of the study revealed that a substantial amount of antioxidant activity is provided by the phenolic compounds present in these medicinal plants.

Rios and Recio. (2005) evaluated the past, present and future of therapeutic plants, both as possible antimicrobial medicine as well as a foundation for innate mixtures that perform as novel anti-infection representatives. They reported that in the earlier periods, the investigation for innovative anti-infection agents has engaged numerous researchers to ethnopharmacology. In literature, one discovers a wide-ranging criteria. Some emphasize on finding out the antimicrobial potential of therapeutic plants used as traditional medicine, oils or bioactive compounds like flavonoids, alkaloids etc. While others focus on green and biosynthesis of nanoparticles from plant material and many more are interested in establishing the role of these biosynthesized nanoparticles as anti-bacterial, anti-fungal, anti-oxidant, cytotoxic and phyto-toxic material. They suggested that some common concerns must be recognized for studying the antimicrobial potentials of the plants, specially the antibacterial activities of biosynthesized metal reduced nanoparticles, the essential oils and the compounds isolated from the plants. The ultimate importance is the characterization of common factors like medium of growth, plant material, tested microbes and techniques employed.

Awadh et al. (2001) screened ethanolic plant extracts of twenty traditional species used locally by Yemeni healers for the treatment of contagious diseases, for antimicrobial potentials against Gram-positive as well as Gram-negative bacterial strains, along with cytotoxic activities. A variable amount of antibacterial activities
were shown by 14 of the total plant extracts. The partition of highly active extracts was carried out with water and ethyl acetate for the first separation. The ethyl acetate extracted fraction of plant *Lawsonia inermis* was observed to be highly vigorous against the bacterial strains under study. Moreover, the cytotoxic activities were also studied for the ethanolic plant extracts of all the 20 tested plants on FL-cells by means of the neutral red assay. Ethanolic extracts of *Tribulus terrestris, Chenopodium murale, Calotropis procera, Pulicaria orientalis* and *Withania somniferum* revealed an outstanding activity.

Ahmad et al. (1998) used several opportunistic pathogenic microbes to study the antibacterial activities of 82 different Indian therapeutic plant species being used as traditional medicines. For this purpose, aqueous, hexane and alcoholic extracts of plants were prepared and analyzed by the help of agar well diffusion method at a concentration of 200 mg/ml. The outcomes showed that out of total 82 plants, 56 presented antimicrobial potential against one or more tested pathogens. Extracts of 5 plants exhibited stronger activities when compared to other 51 plants with moderate activities. The alcoholic plant extracts were found highly significant as compared to its corresponding hexane and aqueous extracts. On the whole, only alcoholic plant extracts of *Terminalia chebula, Plumbago zeylanica, Emblica officinalis, Holarrhena antidysenterica* and *Terminalia belerica* were found with fairly remarkable potential against the test strains.
III. MATERIALS AND METHODS

Plants Identification and Collection

The current research programme was carried out at ‘The Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar Pakistan, International Centre for Chemical and Biological Sciences, Hussain Ebrahim Jamal Research institute (ICCBS), The University of Karachi Pakistan and Bioactive Natural Products and Phytoceuticals Laboratory, Department of Horticulture, Michigan State University (USA). The experimental Plant material was identified by Prof. Dr. Farrukh Hussain, plant taxonomist at Department of Botany, University of Peshawar (KPK). After documentation, the very first step was the bulk collection of the plants from mountainous regions of Swat Valley.

Preparation of Methanolic Crude Extracts of Leaves, Stems and Roots

The whole plant was thoroughly washed first with tap water, followed by distilled water inorder to take out dirt particles. Plant materials were kept in dark shade for two weeks for proper drying. Dried plant materials was grinded using grinding mill (Thomas Scientifc, USA). The following procedure was adopted for the preparation of methanolic crude extracts. Analytical grade methanol was poured to the ground plants material (leaves, shoots and roots) and left for about 10 days with sporadic shaking for the dissolution of bioactive chemical compounds. After 10 days, the soaked plant materials were carefully filtered using Whatman No. 1 filter paper. The same technique was repeated three times for maximum recovery of active chemical compounds present in the plants. The pooled materials were dried at 45°C using rotary evaporator. The resultant semi solid plant crude extract was kept in water bath at 35°C for complete drying. The dried material was carefully weighed and distributed into two different parts. One of the part (10g) was used as methanolic crude and the remaining 60g was kept for fractionation with different solvents. Similar steps were repeated for all the collected plants samples.
**Fractionation of Methanolic Extract**

The dried plant crude material was dissolved in 300 ml of distilled water and dispensed to separatory funnel for further partitioning using different solvents. Three hundred ml of n-hexane was added to the separatory funnel. The resulting solution was slowly shaken and left as it for 15-20 minutes till the formation of two separate layers. The upper layer of n-hexane was collected while the aqueous portion was again extracted with 300 ml of n-hexane. The entire process was repeated thrice. All the fractions obtained from n-hexane were combined, filtered and dried through rota evaporator under reduced pressure to get a semi-solid fraction which was left at room temperature for complete drying. Similar procedure of fractionation was used for butanol and ethyl acetate extracted samples. To sum up, the aqueous phase was combined and dried separately. Five different solvent extracted fractions were obtained at the end of the whole procedure.

**Protocol for Antimicrobial activity**

The antibacterial activity of different extracts of *Viola pilosa* and *Skimmia laureola* and their respective NPs was carried out by the following procedures.

A) **Preparation of Culture Media and Basal Plates**

The methods of Bakht et al. (2011) was used for culture and growth of the microbes under study. A specific amount of nutrient broth (3.25g/250ml) and nutrient agar (7g/250ml) were prepared. The flasks having media were tightly sealed with cotton plugs and autoclaved at 121°C and 15 psi pressure for about 15 minutes. The nutrient agar media was then taken to laminar flow hood and carefully transferred into sterilized petri plates. The petri dishes were allowed to solidify for about twenty minutes and then incubated for next 24 hours at a temperature of 37°C. After incubation, only uncontaminated petri plates were used for antimicrobial assay.

B) **Disc diffusion susceptibility Assay**

Disc diffusion technique was used for analysis of antibacterial potential of various solvent extracted plant fractions and their respective nanoparticles, against diverse bacterial strains as described earlier by Bauer et al. (1966). Fresh cultures of
bacterial strains were used for the experiment. After dilution of broth media containing microbial strains (a standardized inoculums $1-2 \times 10^7$ CFU ml-$^{-1}$ 0.5 McFarland Standard), about 50 μl of microbe culture was used for inoculation and absorption on media plates. Sterile Whatman No. 1 grade filter paper (6 mm) disks were fixed on the cultured petri dishes with the help of autoclaved forceps. Methanolic crude extract followed by aqueous, n-hexane, n-butanol and ethyl acetate fractions dissolved in 1.5ml of DMSO ($1\text{mg}/6\mu\text{l}$) were applied to the discs in a concentration of 0.5, 1 and 2 mg in 6, 12 and 18 μl volumes. The inoculated plates were properly labeled, wrapped and left for incubation at 37 °C for 18 - 24 hours. DMSO was applied as negative control while Azithromycin was taken as positive control with a concentration of 50μg/6μl. Lastly, the zones of growth inhibition in millimeters were measured around the discs.

**Antifungal Activity Protocol**

Potato dextrose agar media was used for culturing the fungal strains in order to test the anti-fungal activity of the different plant fractions and their nanoparticles. Fungal strains were isolated under aseptic conditions from different sources and grown on PDA at 25°C until proper sporulation. The spores were collected into potato dextrose broth. An adequate amount of commercial potato dextrose agar media was prepared, autoclaved and transferred into sterilized petri plates. The petri dishes were left for solidification followed by making of wells in the media discs. Different plant extracts, nanoparticles of extracts and fungal discs were placed to the wells in concentration of 6μl, 12μl and 18μl. The Fungal petri dishes were kept aside at room temperature for appropriate dispersal of spores and then incubated at a temperature of 25°C for next 96 hrs. Finally the plates were inspected for antifungal activity and growth reduction at all the three concentrations (Igbinosa et al., 2009).

**Microorganisms Used for Antimicrobial and Antifungal Assay**

Different bacterial and fungal strains were used for the Antibacterial and antifungal potential of solvent extracted samples and their nanoparticles (Table 1 and Table 2).
DPPH Radical Scavenging/Antioxidant Activity

The methods of Mensor et al. (2001) were implied to study the DPPH free radical scavenging/antioxidant activities of the fractioned extracts and their respective nanoparticles. Stocks of the fractioned extracts were diluted with methanol to get the concentrations of 250, 125, 50 and 25μg ml⁻¹. Approximately 2.5ml of extract solution was mixed with 1.2 ml of a 0.3 mM DPPH in falcon tubes. The falcon tubes were covered with aluminum foil and kept aside to react at room temperature for 30 minutes. The UV absorbance of the resultant mixture was determined at 518 nm and converted to percentage antioxidant activity (AA %) using the following formula.

\[
\text{AA}\% = 100 - \frac{[(\text{Abs sample} - \text{Abs blank}) \times 100]}{\text{Abs control}}
\]

Where;
Blank = Methanol (1.0 ml) + plant extract solution (2.5 ml).
Negative control = (1 ml of 0.3 mM DPPH + methanol (2.5 ml)

Qualitative and Phytochemical analysis

Different protocols were followed for the confirmation of bioactive natural components in the test plants.

i) Confirmatory Test of Tannins

The existence of tannins in the plant extracts was tested. For this purpose, 0.5 mg of each fraction was dissolved in 1ml double distilled water. Addition of 2-3 drops of Ferric chloride solution to the mixture resulted in the color change. The appearance of blue color was observed for Gallic Tannins whereas greenish black color confirmed the presence of Catecholic tannins (Iyengar, 1995).
Table 1: Bacterial Strains used for evaluation of antibacterial potential of the different extracts.

<table>
<thead>
<tr>
<th>Microbial Species</th>
<th>Gram strain type</th>
<th>Details of the Microbial strains used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumonia</td>
<td>Negative</td>
<td>Clinical isolate obtained from the Department of Microbiology, Quaid-I- Azam University Islamabad Pakistan</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Negative</td>
<td>ATCC # 9721</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Positive</td>
<td>ATCC # 6538</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>Positive</td>
<td>Clinical isolate obtained from the Department of Microbiology, Quaid-I- Azam University Islamabad Pakistan</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Negative</td>
<td>ATCC # 25922</td>
</tr>
<tr>
<td>Xanthomonas campestris</td>
<td>Negative</td>
<td>ATCC # 33913</td>
</tr>
</tbody>
</table>

Table 2: Fungal Strains used for evaluation of antifungal potential of the different extracts.

<table>
<thead>
<tr>
<th>Name of the specie</th>
<th>Details of the specie used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>ATCC #10231 obtained from the Department of Plant Pathology, The University of Agriculture Peshawar, Pakistan</td>
</tr>
<tr>
<td>Paecilomyces lilacinus</td>
<td>ATCC # 36010 obtained from the Department of Plant Pathology, The University of Agriculture Peshawar, Pakistan</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>ATCC #11414 obtained from the Department of Plant Pathology, The University of Agriculture Peshawar, Pakistan</td>
</tr>
<tr>
<td>Rhizopus oryzae</td>
<td>ATCC # 20344 obtained from the Department of Plant Pathology, The University of Agriculture Peshawar, Pakistan</td>
</tr>
<tr>
<td>Alterneria solani</td>
<td>ATCC #58177 obtained from the Department of Plant Pathology, The University of Agriculture Peshawar, Pakistan</td>
</tr>
<tr>
<td>Curvularia lunata</td>
<td>ATCC # 38850 obtained from the Department of Plant Pathology, The University of Agriculture Peshawar, Pakistan</td>
</tr>
</tbody>
</table>
ii) **Test for Alkaloids**

The presence of alkaloids was determined through Mayer’s Test. For this purpose, 50 mg of fractioned extract was mixed with 10 ml dilute hydrochloric acid. About 2-3 drops of Mayer’s reagent. Appearance of white/creamy precipitate demonstrated the occurrence of alkaloids (Tiwari et al., 2011).

iii) **Confirmatory Test for Sterols**

Salkowski experiment was used for the verification of Phytosterols. Three mg of plant fraction was dissolved in 2 ml of chloroform followed by the addition of 2 ml concentrated sulphuric acid to the sides of the test tube. Appearance of bright red color in the chloroform layer evidenced the presence of sterols (Tiwari et al., 2011).

iv) **Saponins Test**

Half gram of the plant fraction dissolved in 3ml of distilled water was vigorously shaken with 10mg of Sodium bicarbonate for 5 min. The formation of persistent foam indicated the presence of saponins (Harborne, 1973).

v) **Carbohydrates Confirmation Test**

Carbohydrate in the extract was confirmed through Fehling’s Test. Hundred mg of the extract was dissolved in 5 ml of distilled water and filtered through whatman.1 filter paper. After filtration, one ml of the filtrate was boiled with 1ml each of Fehling’s solution A and solution B. The formation of red precipitate specified the existence of sugar (Tiwari et al., 2011).

vi) **Confirmatory Test for Flavonoids**

About 0.2 g of extract was dissolved in 5ml of NaOH solution followed by the addition of few drops of concentrated HCl. The resultant solution turned colorless that evidenced the presence of flavonoids (Siddiqui and Ali, 1997).
vii) Spot Test of Fixed Oils and Fats

Fixed oils in the extracts were detected through Spot Test. For this purpose, a small amount of the plant fraction was sandwiched between filter papers. Oil stains on the two papers revealed the presence of fixed oils and fats.

viii) Confirmatory test for proteins

Biuret experiment was chosen to find out the existence of Proteins in the plant extracted fractions. About 0.020g of fractioned extract was dissolved in 10 ml of distilled water followed by filtration and addition of 1ml 4% CuSO₄ solution to the filtrate. Appearance of violet pink color confirmed the existence of Proteins (Chulet et al., 2010).

Insecticidal Activity of Plant Extracts

The insecticidal potential of different plant extracts was carried out using two insect species Tribolium castaneum and Sitophilus oryzae. Permethrin was used as a standard insecticide for the test. The following steps were followed for the activity.

A) Formulation of Plant Test Fractions

For making the test fractions, different plant extracts were dissolved in methanol to achieve the ultimate dilution of 5mg/ml, 25mg/ml and 125 mg/ml.

B) Rearing of the Insect Species

Two different stored grain pest species Tribolium castaneum and Sitophilus oryzae were obtained from The Department of Entomology, The University of Agriculture Peshawar Pakistan. Fresh grains were infected with these pests. Upon completion of life cycle, about 5g of the infected grains containing insect eggs were mixed with some more fresh grains. The eggs began to hatch into small insects after seven days of infestation. One day old pest cultures were selected for the experiment according to the methods of Rehmanullah et al. (2012).
C) **Steps of Insecticidal Activity**

Round cut filter papers of 90mm size were autoclaved and fixed in petri dishes. About 5ml of each test sample was loaded to the petri dishes and kept in open air for next 24 hours for complete evaporation of methanol. The experiment was repeated in three replicates with addition of 30 insects to each plate (10 insects/rep). The plates with insects were kept in growth chamber for 24 hours at 27°C. The readings were recorded after three days, in accordance to the technique already used by Rehmanullah et al. (2012). A statistical analysis program IBM SPSS statistics 20 was used for the analysis of survival data.

**Phytotoxic Activity of Solvent Extracted Plant Extracts**

Stock solutions were made by dissolving 0.025mg of each plant extract in 3ml of methanol separately. Three concentrations of 6, 12 and 18 µl were prepared from the stock extract solutions in falcon tubes and kept in open air for 24 hours to evaporate methanol. The next day, sterilized flasks containing three fronded plants of *Lemna minor* were filled with 20 ml of slightly basic pH enriched media. These flasks were kept in growth chamber for seven consecutive days (at 60% humidity, 30°C and 9000 flux light intensity). The fronds of the plant were measured on the eighth day of experiment. Parquet was considered as a positive control. A statistical analysis program IBM SPSS statistics 20 was used for the survival rate calculation and a formula described by Ramanullah et al. (2012) was chosen for the examination of percent growth of plant.

\[
\% \text{ Growth inhibition} = \frac{\text{Fronds in sample}}{\text{Fronds in negative control}} \times 100
\]

**Biosynthesis of Silver/Gold Nano particles (Ag/AuNPs)**

Silver/Gold Nanoparticles (Ag/AuNPs) were synthesized according to the following protocol
1) **Washing of the glass Vessels**

All the glass ware used in the creation of Ag/AuNPs was thoroughly washed away by using de-ionized water and Aqua regia (Mixture of Hydrochloric and Nitric acid, ratio of 3:1) to remove particles of metal impurities.

2) **Preparation of Plant Extract**

For the biogenic production of Au/AgNPs, 50mg of dried and grinded stems of *Viola pilosa* and *Skimmia laureola* were soaked in 100ml of double distilled water for 24 hours to get the pure plant extract for the synthesis of nanoparticles.

3) **Method of Biosynthesis**

AuCl₃/ AgNO₃ solutions (0.1 mM) were prepared and kept in amber colored bottles. Different volumes of extracts were taken in small glass vials separately. The solution of 0.1 mM AuCl₃/ AgNO₃ solution was poured drop wise to the plant extracts with sporadic stirring at room temperatures. The color alteration of the mixture was monitored from time to time and then the glass vials were incubated at room temperature for next 48 hours. The variation in color of the plants extracts from pale yellow to brownish specified the formation of silver nanoparticles (Ateeq et al., 2015). The occurrence of pink and purple colors showed the production of gold nanoparticles. The plant extracted solutions for further confirmation of synthesis were exposed to UV-Vis spectrum. These nanoparticles were further subjected to characterization, antifungal, antimicrobial and antioxidant activities.

**Nanoparticles Characterization**

The following techniques were adopted for the characterization of the newly synthesized nanoparticles.

1. **UV- Vis Spectroscopy**

UV-Vis Spectroscopy of the synthesized silver/gold nanoparticles formed was documented through dual beam spectrophotometer (UV Probe, Shimadzu, version 2.42, Japan) (Ateeq et al., 2015). The highest peaks confirmed the separation of Ag/Au nanoparticles.
2. **Scanning Electron Micrographic Study**

   To explicate the shape and size of the Ag/AuNPs, a high resolution Scanning Electron Microscopic study was carried out. Liquid extract samples were treated with ultra clean carbon coated copper grid and permitted to vaporize at ambient conditions for advanced study.

3. **X-Ray diffraction or XRD analysis**

   The powdered Ag/AuNPs were coated evenly onto a glass slide to attain a random distribution. The nature and particle size of Ag/AuNPs along with their unit cell dimension was determined by X-Ray diffracto-meter.

4. **FT-IR or Fourier transform infrared spectroscopy**

   FT-IR spectroscopy was also carried out by FT-IR spectrophotometer (Shimdzu IR 460). The dried nanoparticles were grinded and subjected to FT-IR analysis for the determination of various functional groups (Dubey et al., 2013).

**Consideration of Biosafety Level**

   Proper bio-safety procedures were observed from solvent extraction to the dumping of materials. Entrance to the laboratory was restricted to only authorized and appropriately dressed personals. Working zone was properly disinfected with 70% ethanol and spirit each time before and after working in the lab. For complete insurance of bio-safety, all the tests were performed in LFU and fume hood.

**Statistical Analysis of Data**

   Each technique of the research was performed in three replicates. MSTAT - C was used for the interpretation of the statistical data. The level of significance at p<0.05 was measured by LSD (Steel et al., 1997).
IV. RESULTS

OBJECTIVE 1:

Biogenic synthesis of gold nanoparticles (AuNPs)

A solution of .1 mM of AuCl$_3$ was formulated for the bioinspired production of gold nanoparticles. The extracts of plants were observed optically and were then confirmed by the help of UV-Vis Spectrophotometric system. The UV-Vis Spectrophotometer detects a resonance which is produced by the collective vibration of the gold nanoparticles and light waves and thus is a proof of nanoparticles formation.

Optimization levels for the creation of gold Nano-Particles:

Various changes ranging from concentration of fractions and synthesis to the UV–Vis spectra were adopted during the course of the development of the proper protocols for successful production of gold nanoparticles from the crude methanolic and aqueous fractions of *Viola pilosa* & *Skimmia laureola*. The optimization of the planned protocols for the production of nano-particles from the crude methanolic and aqueous fractions of the roots and shoots of *Viola pilosa* & *Skimmia laureola* leaves and stem resulted in either zero or abnormal peaks. Very low concentrations of gold chloride and plant extract fractions were used for the preliminary protocols designed for the production of crude and aqueous extracted gold NPs. No change in color and no definite peaks were observed for the non-stirring extracts when scanned with Spectrophotometer. The modification in the ratios of gold chloride, crude methanolic and aqueous fractions of *Viola pilosa* & *Skimmia laureola* in stirring protocol lead to the gradual creation of the gold nanoparticles. It was clearly observed that an increase in the ratio of the plant extracted fraction and AuCl$_3$ resulted in denser pink color of the solutions which was a sign of synthesis of a good amount of nanoparticles. The standard for selecting a healthier ratio was dependent on the shape of the curve on UV–Vis analysis. The ratio with a finer and sharper curve was designated as the optimum peak ratio.
The final optimized extract ratios chosen for advance studies were 4:1 (4ml of \(\text{AuCl}_3\) (0.1mM) and 1ml of plant extract) for \textit{Viola} crude root extracts, \textit{Viola} shoots crude and \textit{Viola} shoots aqueous extracts respectively. However, 2:1 (2ml of \(\text{AuCl}_3\) (0.1mM) and 1ml of plant extract) for the \textit{Viola} root aqueous extracts. Similarly, a ratio of 1:1 (1ml of \(\text{AuCl}_3\) (0.1mM) and 1ml of plant extract) was observed for the \textit{Skimmia} stem aqueous extract, a ratio of 4:1 (4ml of \(\text{AuCl}_3\) (0.1mM) and 1ml of plant extract) was observed for stem crude and leaves aqueous extracts of \textit{Skimmia laureola} and 3:1 (3ml of \(\text{AuCl}_3\) (0.1mM) and 1ml of plant extract) was observed for the \textit{Skimmia} leaves crude extract. The results indicated a separate color for each type of nanoparticles synthesized from crude methanolic and aqueous fractions of \textit{Viola pilosa} & \textit{Skimmia laureola}.

**UV-Vis confirmation of \textit{Viola} roots crude AuNPs**

The UV spectrum shown in Figure 1 characterizes the AuNPs synthesized from the ratio of the 0.1 mM \(\text{AuCl}_3\) solution and \textit{Viola pilosa} roots crude extract. The obtained spectrum specified that the highest peak was observed for the combination of 4ml of \(\text{AuCl}_3\) solution and 1ml of the plant crude root extract (4:1). Final analysis of the curve for \textit{Viola} root crude revealed an absorbance of 0.099 of the nanoparticles solution at the highest peak of 537.72 nm wavelength. The optimum range for the AuNPs is between 500 - 600 nm. The wavelength of 537.72 nm comes within the required range hence confirming the production of the gold nanoparticles.

**UV-Vis confirmation of \textit{Viola} roots aqueous AuNPs**

Figure 2 signifies the AuNPs formed by the combination of the 0.1 mM \(\text{AuCl}_3\) and \textit{Viola pilosa} roots aqueous extract solutions. The spectrum curve indicated that the highest peak was noticed for 2ml of \(\text{AuCl}_3\) solution and 1ml of the \textit{Viola} root aqueous extract (2:1). As per results, the highest sharp peak was observed at 2:1 and was thus used for the further analysis of nanoparticles. The results of spectra analysis for \textit{Viola} root aqueous extracts exposed an absorbance (0.1) of the nanoparticles extract solutions at the highest wavelength of 539.37 nm. The 539.37 nm fits into the wavelength of (500-600 nm) thus approving the presence of the AuNPs in the extracted solution.
Figure 1: UV-Vis confirmation of Viola roots crude AuNPs formed by combination of 4ml of AuCl₃ solution (0.1mM) and 1ml Viola roots crude solution presenting AuNPs peak at 537.72 nm.

Figure 2: UV-Vis confirmation of Viola roots aqueous AuNPs formed by combination of 2ml of AuCl₃ solution (0.1mM) and 1ml Viola roots crude solution presenting AuNPs peak at 539.37 nm.
UV-Vis confirmation of Viola shoot crude AuNPs

The UV spectrum shown in Figure 3 characterizes the AuNPs synthesized from the ratio of the 0.1 mM AuCl$_3$ solution and Viola pilosa shoot crude extract. The obtained spectrum specified that the highest peak was observed by the combination of 4ml of AuCl$_3$ solution and 1ml of the plant crude root extract (4:1). Final analysis of the curve for Viola shoot crude revealed an absorbance of 0.496 of the nanoparticles solution at the highest peak of 531.78 nm wavelength. The optimum range for the AuNPs is between 500-600 nm. The wavelength of 531.78 nm comes within the required range hence confirming the creation of the gold nanoparticles.

UV-Vis confirmation of Viola shoots aqueous AuNPs

Figure 04 signifies the AuNPs formed by the ratio of the 0.1 mM AuCl$_3$ solution and Viola pilosa shoots aqueous extract. The spectrum curve indicated that the maximum peak was noticed for 4ml of AuCl$_3$ solution and 1ml of the Viola shoot aqueous extract (4:1). As per results, the highest sharp peak was observed at 4:1 and was thus used for the further analysis of nanoparticles. The results of spectra analysis for Viola shoot aqueous extracts exposed an absorbance (0.362) of the nanoparticles extract solutions at the highest wavelength of 541.8 nm. The 541.81 nm fits into the wavelength of (500 - 600 nm) thus approving the presence of the AuNPs in the extract solution.

Stability analysis of AuNPs of Viola pilosa roots and shoots

After synthesis of nanoparticles, the study was proceeded to check the stability of AuNPs of Viola pilosa roots and shoots by passing them through salt and heat stress. The UV-Vis spectrophotometer was applied to find out the distinctive effects of heat and salt stress parameters on the gold nanoparticles.
Figure 3: UV-Vis confirmation of *Viola* shoot crude AuNPs formed by combination of 4ml of AuCl$_3$ solution (0.1mM) and 1ml *Viola* shoot crude solution presenting AuNPs peak at 531.78 nm.

Figure 4: UV-Vis confirmation of *Viola* shoot aqueous AuNPs formed by combination of 4ml of AuCl$_3$ solution (0.1mM) and 1ml *Viola* shoot aqueous solution presenting AuNPs peak at 541.81 nm.
Heat stress stability analysis of Viola pilosa roots and shoots AuNPs

The crude and aqueous extracted nanoparticles from roots and shoots of Viola pilosa were subjected to different degrees of temperatures. The results showed that the stability of nanoparticles was inversely proportional to the increasing temperatures. The results of Figures 5 show that the nanoparticles in case of Viola root crude, Viola root aqueous, Viola shoot crude and Viola shoot aqueous extracted gold nanoparticles were found highly stable at the temperature ranges of 25°C - 50°C giving the highest peak at an absorbance level of 0.438. The study proved that rise in temperature reduced the stability of the gold nanoparticles in a temperature ranging from 50°C - 60°C thus lowering the peak of the tested samples. However, the analysis of Viola crude and aqueous extracted AuNPs also indicated that the nanoparticles were totally unstable and were damaged at a temperature range of 60°C - 100°C presenting no absorbance through spectrophotometer.

Salt stress stability analysis of Viola pilosa roots and shoots AuNPs

Sodium chloride was used as a standard salt to inspect the influence of salt stress on the gold nanoparticles of Viola pilosa roots and shoots extracts. The experimental protocol started with preparation of three different concentrations of salt (0.1mM, 0.5mM and 1M) which were added separately to the nanoparticles solutions. The results of experiment recommended that an inverse relationship was found between the concentration of Sodium chloride and degree of stability of nanoparticles. An increase in the NaCl concentration resulted in the reduced stability of the AuNPs. The highest peak in the Figure 6 presents the pure AuNPs solution with no salt in it. The second highest peak represents the decrease in stability when 0.1mM salt solution was added to the nanoparticles. Addition of more salt concentrations (0.5mM and 1M NaCl) resulted in instability of the gold nanoparticles. The UV-spectroscopic absorption and sharper peaks clearly show the negative effects of salt stress on stability of the AgNPs. Moreover, it was also noticed that the molar (1M) concentration of NaCl adversely effected the AgNPs as compared to the millimolar concentrations (0.1mM and 0.5mM). Amongst the tested concentrations, the nanoparticles were found much stable at 0.1mM, comparatively moderately stable at 0.5mM and least stable at a salt concentration of 1M.
Figure 5: UV-Vis spectrum comparison of heat stability of *Viola* shoots and roots AuNPs: (a) *Viola* root crude AuNPs, (b) *Viola* root aqueous AuNPs, (c) *Viola* shoot crude AuNPs, (d) *Viola* shoot aqueous AuNPs.
Figure 6: UV-Vis spectrum comparison of salt stress stability of *Viola* shoots and roots AuNPs: (a) *Viola* root crude AuNPs, (b) *Viola* root aqueous AuNPs, (c) *Viola* shoot crude AuNPs, (d) *Viola* shoot aqueous AuNPs.
Bioinspired formation of AuNPs of *Skimmia* stem and leaves extracts

The visible change in color and sharper peaks of UV-Vis Spectroscopic inquiry specified the synthesis of gold nanoparticles. The solution for green synthesis of nanoparticles was prepared by using 25 ml of .1 Mm AuCl$_3$ solution and 25 ml (25%) of *Skimmia laureola* stem and leaves crude and aqueous extracts. The mixture was incubated, for next 30 minutes at a room temperature with a constant shaking, which resulted in the change of colorless extract to a pinkish solution that clearly indicated the production of gold nanoparticles.

**UV-Vis confirmation of *Skimmia laureola* stem crude AuNPs**

The UV spectrum shown in Figure 7 characterizes the AuNPs synthesized from the ratio of the 0.1 mM AuCl$_3$ solution and *Skimmia laureola* stem crude extract. The obtained spectrum specified that the highest peak was observed by the combination of 4ml of AuCl$_3$ solution and 1ml of the plant crude root extract (4:1). Final analysis of the curve for *Skimmia* stem crude revealed an absorbance of 0.797 of the nanoparticles solution at the highest peak of 536.20 nm wavelength. The optimum range for the AuNPs is between 500 nm – 600 nm. The wavelength of 536.20 nm comes within the required range hence confirming the creation of the gold nanoparticles.

**UV-Vis confirmation of *Skimmia* stem aqueous AuNPs**

Figure 8 signifies the AuNPs formed by the ratio of the 0.1 mM AuCl$_3$ solution and *Skimmia* stem aqueous extract. The spectrum curve indicated that the highest peak was noticed for 1ml of AuCl$_3$ solution and 1ml of the *Skimmia* stem aqueous extract (1:1). As per results, the highest sharp peak was observed at 1:1 and was thus used for the further analysis of nanoparticles. The results of spectra analysis for and *Skimmia* stem aqueous extracts exposed an absorbance 0.200 of the nanoparticles extract solutions at the highest wavelength of 537.72 nm. The 537.72 nm fits into the wavelength of (500 nm – 600 nm) thus approving the presence of the AuNPs in the extract solution.
Figure 7: UV-Vis confirmation of *Skimmia* stem crude AuNPs formed by combination of 4ml of AuCl$_3$ solution (0.1mM) and 1ml *Skimmia* stem crude solution presenting AuNPs peak at 536.20 nm.

Figure 8: UV-Vis confirmation of *Skimmia* stem aqueous AuNPs formed by combination of 1ml of AuCl$_3$ solution (0.1mM) and 1ml *Skimmia* stem aqueous solution presenting AuNPs peak at 537.72 nm.
UV-Vis confirmation of *Skimmia laureola* leaves crude AuNPs

The UV spectrum shown in Figure 9 characterizes the AuNPs synthesized from the ratio of the 0.1 mM AuCl$_3$ solution and *Skimmia laureola* leaves crude extract. The obtained spectrum specified that the highest peak was observed by the combination of 3ml of AuCl$_3$ solution and 1ml of the plant crude leaves extract (3:1). Final analysis of the curve for *Skimmia* leaves crude revealed an absorbance of 0.300 of the nanoparticles solution at the highest peak of 584.76 nm wavelength. The optimum range for the AuNPs is between 500 nm – 600 nm. The wavelength of 584.76 nm comes within the required range hence confirming the formation of the gold nanoparticles.

**UV-Vis confirmation of *Skimmia* leaves aqueous AuNPs**

Figure 10 signifies the AuNPs formed by the ratio of the 0.1 mM AuCl$_3$ solution and *Skimmia* stem aqueous extract. The spectrum curve indicated that the uppermost peak was noticed for 4ml of AgNO$_3$ solution and 1ml of the *Skimmia* leaves aqueous extract (4:1). As per results, the highest sharp peak was observed at 4:1 and was thus used for the further analysis of nanoparticles. The results of spectra analysis for and *Skimmia* leaves aqueous extracts exposed an absorbance 0.099 of the nanoparticles extract solutions at the highest wavelength of 556.91 nm. The 556.91 nm fits into the wavelength of (500 nm – 600 nm) thus approving the presence of the AuNPs in the extract solution.

**Stability Analysis**

**Heat stress stability analysis of *Skimmia laureola* stem and leaves AuNPs**

The crude and aqueous extracted nanoparticles from stem and leaves of *Skimmia laureola* were subjected to different degrees of temperatures. The results showed that the stability of nanoparticles was inversely proportional to the increasing temperatures. The results of Figure 11 show that the nanoparticles in case of *Skimmia* stem crude, *Skimmia* stem aqueous, *Skimmia* leaves crude and *Skimmia* leaves aqueous extracted gold nanoparticles were found highly stable at the temperature ranges of 20°C - 40°C giving the highest peak at an highest level of absorbance .
study proved that an increase in temperature reduced the stability of the gold nanoparticles in a temperature ranging from 40°C - 60°C thus lowering the peak of the tested samples. However, the analysis of *Skimmia* crude and aqueous extracted AuNPs also indicated that the nanoparticles were totally unstable and were damaged at a temperature range of 60°C - 100°C presenting no absorbance through spectrophotometer.

**Salt stress stability analysis of *Skimmia laureola* stem and leaves AuNPs**

The highest peak in the figure 12 presents the pure AuNPs solution with no salt in it. The second highest peak represents the decrease in stability when 0.1mM salt solution was added to the nanoparticles. Addition of more salt concentrations (0.5mM and 1M NaCl) resulted in instability of the gold nanoparticles. The UV-spectroscopic absorption and sharper peaks clearly show the negative effects of salt stress on stability of the AuNPs. Moreover, it was also noticed that the molar (1M) concentration of NaCl adversely effected the AuNPs as compared to the milli Molar concentrations (0.1mM and 0.5mM). Amongst the tested concentrations, the nanoparticles were found much stable at 0.1mM, comparatively moderately stable at 0.5mM and least stable at a salt concentration of 1M.

**X-Ray Diffraction (XRD) study of *Viola* and *Skimmia* AuNPs extracts:**

The biologically formed gold nanoparticles of the *Viola* and *Skimmia* plants extracts were then subjected to XRD technique for the ratification of the crystalline structure. X-ray diffraction is a well-known technique used for the characterization of crystallographic nature, size dimension, and ideal orientation in solid polycrystalline and powdered samples. This technique is always favored for categorization of indefinite crystalline structures. The XRD analysis pattern indicated the crystalline nature of the gold nanoparticles.
Figure 9: UV-Vis confirmation of *Skimmia* leaves crude AuNPs formed by combination of 3ml of AuCl$_3$ solution (0.1mM) and 1ml *Skimmia* leaves crude solution presenting AuNPs peak at 584.76 nm.

![Absorbance vs Wavelength](image1.png)

Figure 10: UV-Vis confirmation of *Skimmia* leaves aqueous AuNPs formed by combination of 4ml of AuCl$_3$ solution (0.1mM) and 1ml *Skimmia* leaves aqueous solution presenting AuNPs peak at 556.91 nm.

![Absorbance vs Wavelength](image2.png)
Figure 11: UV-Vis spectrum comparison of heat stability of *Skimmia laureola* stem and leaves AuNPs (a) *Skimmia* stem crude AuNPs, (b) *Skimmia* stem aqueous AuNPs, (c) *Skimmia* leaves aqueous AuNPs.
Figure 12: UV-Vis spectrum comparison of heat stability of *Skimmia laureola* stem and leaves AuNPs (a) *Skimmia* stem crude AuNPs, (b) *Skimmia* leaves crude AuNPs, (c) *Skimmia* leaves aqueous AuNPs.
X-Ray Diffraction (XRD) analysis of Viola shoot crude AuNPs extracts:

The demonstrative X-Ray diffraction configuration of the powdered gold nanoparticles of Viola shoot crude is presented in Figure 13. XRD study indicated three distinctive deflection peaks within the 2 theta range at angles of 13.07°, 27.76° and 38.32° respectively, acting to be indexed in the diverse planes (110), (310), and (312) of the facets of gold nanoparticles. These planes of gold nanoparticles were in correspondence to those present in database of the Joint Committee on Powder Diffraction Standards, USA (JCPDS no. 00-004-0784), approving the pure crystallographic centrosymmetric structure of the synthesized nanoparticles. The results of the profile declared that the peak at 312 position was found to be the most dominating diffraction pattern amongst all the other peaks of the analyzed nanoparticles. The full width half maximum determination of the most intense peak (312), by the help of Sherrer equation was applied for the typical size calculation of the newly formed AuNPs, which was appeared to be 27.12 nm. The sharpness of the peak undoubtedly specified that the nanoparticles were present in nano region.

X-Ray Diffraction (XRD) study of Viola shoot aqueous AuNPs extracts:

Figure 14 shows the results of the XRD study carried out for the evaluation of the crystalline configuration of the gold nanoparticles produced from the aqueous extract of Viola shoots. Examination of X-Ray diffraction peaks specified distinctive peaks at two theta values of 28.41°, 37.99° and 40.55° parallel to (100), (101) and (110) surfaces of gold NPs respectively. The index of Bragg’s reflections was done on the source of face centered cubic Au structure. The dimension of the facets exposed the crystalline centrosymmetric nature of the synthesized gold nanoparticles. Determining FWHM for the strongest reflection of (100) on XRD data by using Sherrer equation proposed an normal size of 26.43 nm.

X-Ray Diffraction (XRD) study of Viola root crude AuNPs extracts:

The X-Ray diffraction conformation of the powdered gold nanoparticles of Viola roots crude is presented in Figure 15. XRD study indicated six distinctive deflection peaks within the 2 theta range at angles of 8.59°, 28.08°, 38.32°, 64.84° and 77.94° respectively, acting to be indexed in the diverse planes (002), (106), (107), (205), (306) and (400) of the facets of gold nanoparticles. These planes of gold nanoparticles were in correspondence to those present in database of the Joint
Committee on Powder Diffraction Standards, USA (JCPDS no. 00-004-0784), approving the pure crystallographic centrosymmetric structure of the synthesized nanoparticles. The results of the profile declared that the peak at 106 position was found to be the most dominating diffraction pattern amongst all the other peaks of the analyzed nanoparticles. The full width half maximum determination of the most intense peak (106), by the help of Sherrer equation was applied for the typical size calculation of the synthesized AuNPs, which appeared to be 3.65 nm. The sharpness of the peak undoubtedly specified that the nanoparticles were present in nano region. The statistical records also discovered that no peaks for the XRD patterns were merely because of the crystalline contaminations, which showed that the AuNPs were extremely pure in nature.

**X-Ray Diffraction (XRD) study of Viola root aqueous AuNPs extracts:**

Figure 16 illustrates the consequences of the XRD study carried out for the evaluation of the crystalline configuration of the gold nanoparticles produced from the aqueous extract of Viola roots. Examination of X-Ray diffraction peaks specified distinctive peaks at two theta values of 22.02°, 28.39°, 37.99°, 44.37°, 64.82° and 77.94° parallel to (110), (111), (112), (200), (220) and (311) surfaces of gold NPs respectively. The index of Bragg’s reflections was done on the source of face centered cubic Au structure. The dimension of the facets exposed the crystalline centrosymmetric nature of the synthesized gold nanoparticles. Determining FWHM for the strongest reflection of (112) on XRD data by using Sherrer equation proposed an typical size of 16.82 nm. The outcomes also concluded that the sharp Bragg diffraction peaks might be a result of capping agents which are stabilizing the gold nanoparticles.

**X-Ray Diffraction (XRD) study of Skimmia stem crude AuNPs extracts:**

The demonstrative X-Ray diffraction arrangement of the powdered gold nanoparticles of Skimmia stem crude is presented in Figure 17. XRD study indicated five distinctive deflection peaks within the 2 theta range at angles of 32.56°, 37.99°, 44.70°, 64.84° and 77.61° respectively, acting to be indexed in the diverse planes (101), (111), (200), (220),
Figure 13: RD peaks of Viola shoot crude AuNPs

Figure 14: RD peaks of Viola shoot aqueous AuNPs
Figure 15: RD peaks of *Viola* root crude AuNPs

Figure 16: RD peaks of *Viola* root aqueous AuNPs
and (311) of the facets of gold nanoparticles. These planes of gold nanoparticles were in correspondence to those present in database of the Joint Committee on Powder Diffraction Standards, USA (JCPDS no. 00-004-0784), approving the pure crystallographic centrosymmetric structure of the synthesized nanoparticles. The results of the profile declared that the peak at 111 position was found to be the most dominating diffraction pattern amongst all the other peaks of the analyzed nanoparticles. The full width half maximum determination of the most intense peak (111), by the help of Sherrer equation was used for the typical size calculation of the newly produced AgNPs, which was appeared to be 9.91 nm. The sharpness of the peak undoubtedly specified that the nanoparticles were present in nano region.

**X-Ray Diffraction (XRD) study of Skimmia stem aqueous AuNPs extracts:**

Figure 18 shows the results of the XRD study carried out for the evaluation of the crystalline configuration of the silver nanoparticles produced from the aqueous extract of *Skimmia* stem. Examination of X-Ray diffraction peaks specified distinctive peaks at two theta values of 38.01°, 44.71°, 64.80° and 77.59° parallel to (111), (200), (220) and (311) surfaces of gold NPs respectively. The index of Bragg’s reflections was done on the source of face centered cubic Au structure. The dimension of the facets exposed the crystalline centrosymmetric nature of the synthesized gold nanoparticles. Determining FWHM for the strongest reflection of (111) on XRD data by using Sherrer equation proposed a typical size of 10.76 nm.

**X-Ray Diffraction (XRD) study of Skimmia leaves crude AuNPs extracts:**

The X-Ray diffraction confirmation of the powdered gold nanoparticles of *Skimmia* leaves crude is presented in Figure 19. XRD study indicated four distinctive deflection peaks within the 2 theta range at angles of 15.95°, 26.80°, 38.63°, and 45.66° respectively, acting to be indexed in the diverse planes (110), (111), (112), and (120) of the facets of gold nanoparticles. These planes of gold nanoparticles were in correspondence to those present in database of the Joint Committee on Powder Diffraction Standards, USA (JCPDS no. 00-004-0784), approving the pure crystallographic centrosymmetric structure of the synthesized nanoparticles. The results of the profile declared that the peak at 112 position was found to be the most dominating diffraction pattern amongst all the other peaks of the analyzed nanoparticles. The full width half maximum determination of the most intense peak
(112), by the help of Sherrer equation was applied for the typical size calculation of the synthesized AuNPs, which was appeared to be 20.94 nm. The sharpness of the peak undoubtedly specified that the nanoparticles were present in the nano region. The statistical information also discovered that no peaks for the XRD configurations were merely because of the crystalline impurities, which showed that the AuNPs were extremely pure in nature.

**X-Ray Diffraction (XRD) study of Skimmia leaves aqueous AuNPs extracts:**

Figure 20 illustrates the consequences of the XRD study carried out for the evaluation of the crystalline configuration of the gold nanoparticles produced from the aqueous plant extract of Skimmia leaves. Examination of X-Ray diffraction peaks specified distinctive peaks at two theta values of 13.33°, 25.12° and 38.48° parallel to (011), (111) and (123) surfaces of gold NPs respectively. The index of Bragg’s reflections was done on the source of face centered cubic Au structure. The dimension of the facets exposed the crystalline nature of the synthesized gold nanoparticles. Determining FWHM for the strongest reflection of (123) on XRD data by using Sherrer equation proposed a typical size of 21.29 nm. The outcomes also concluded that the sharp Bragg diffraction peaks might be a result of capping agents which are stabilizing the gold nanoparticles.

**Fourier Transformed Infrared (FT-IR) verification of AuNPs:**

The gold nanoparticles (AuNPs) produced from the crude and aqueous extracts of Viola pilosa and Skimmia laureola were subjected to Fourier Transformed Infrared (FTIR) spectroscopic analysis for the documentation of probable functional groups responsible for reduction of gold (Au⁺) ions.

**Fourier Transformed Infrared (FT-IR) spectroscopic study of Viola roots and shoots AuNPs**

The FT-IR spectra of Viola roots (Figure 21) and shoots (Figure 22) methanolic crude extracts proposed that the roots and shoots extracted nanoparticles presented major absorption bands of infrared radiation at wave numbers 3315.6 cm⁻¹, 3316.3 cm⁻¹, 1639.7 cm⁻¹, 1219.6 cm⁻¹ and 1219.7 cm⁻¹ respectively.
Figure 17: RD peaks of *Skimmia* stem crude AuNPs

Figure 18: RD peaks of *Skimmia* stem aqueous AuNPs
**Figure 19:** RD peaks of *Skimmia* leaves crude AuNPs

**Figure 20:** RD peaks of *Skimmia* leaves aqueous AuNPs
By comparing the FTIR range of AuNPs to the FT-IR table values, it was discovered that the infrared radiation absorption band at wave numbers 3315.6 cm\(^{-1}\) and 3316.3 cm\(^{-1}\) present in ranges of nanoparticles were found to be in the spectrum of 3200-3550 when compared to the typical infrared absorption frequencies, thus presenting Phenols. The shift in the band discovered that the \(-\text{O–H}\) functional group holding compounds were generally accountable for reducing the gold (Au) metal. It was also noticed that absorption band at wave numbers 1639.7 cm\(^{-1}\), 1219.6 cm\(^{-1}\) and 1219.7 cm\(^{-1}\) in spectra of nanoparticles were found to be in the range of 1600-1750 and 1200-1300, thus presenting esters. Sharp Peaks at 1639.7 cm\(^{-1}\) represent carbonyl group of esters. \((\text{C} = \text{O})\) and corresponding peak at 1219.6 cm\(^{-1}\) and 1219.7 cm\(^{-1}\) characterizes ether group of esters. \((\text{R–O–R})\).

FTIR spectra analysis of *Viola* roots (Figure 23) and shoots aqueous (Figure 24) extracts proposed that the roots and shoots extracted nanoparticles presented major absorption bands of infrared radiation at wave numbers 3328.0 cm\(^{-1}\), 3300.6 cm\(^{-1}\) and 1639.7 cm\(^{-1}\) respectively. By comparing the FTIR spectrum of AuNPs to the FT-IR table values, it was discovered that the infrared radiation absorption band at wave numbers 3328.0 cm\(^{-1}\) and 3300.6 cm\(^{-1}\) present in spectrum range of nanoparticles were found to be in the range of 3200-3550 when compared to the typical infrared absorption frequencies, thus presenting Phenols. The shift in the band discovered that the \(-\text{O–H}\) functional group holding compounds were primarily in charge of reducing gold (Au) metal. It was also noticed that absorption band at wave numbers 1639.7 cm\(^{-1}\) in spectrum of nanoparticles were found to be in the range of 1600-1750 thus presenting esters. Sharp Peaks at 1639.7 cm\(^{-1}\) represent carbonyl group of esters. \((\text{C} = \text{O})\).

**Fourier Transformed Infrared (FT-IR) spectroscopic study of *Skimmia* stem and leaves AuNPs**

FTIR spectra of *Skimmia* leaves aqueous extract proposed that the leaves extracted nanoparticles presented major absorption bands of infrared radiation at wave numbers 3301.6 cm\(^{-1}\), 1639.7 cm\(^{-1}\) and 1219.5 cm\(^{-1}\) respectively. By comparing the FTIR spectrum of AuNPs to the FT-IR table values, it was discovered that the infrared radiation absorption band at wave number 3301.6 cm\(^{-1}\) present in spectra of
nanoparticles was found to be in the range of 3200-3550 when compared to the typical infrared absorption frequencies, thus presenting Phenols. The shift in the band discovered that the \(-\text{O—H}\) functional group holding compounds were generally accountable for reducing the gold (Au) metal. It was also noticed that absorption band at wave numbers 1639.7 cm\(^{-1}\) and 1219.5 cm\(^{-1}\) in spectra of nanoparticles were found to be in the range of 1600-1750 and 1200-1300, thus presenting esters. Sharp Peaks at 1639.7 cm\(^{-1}\) represent carbonyl group of esters. (C\(\equiv\)O) and corresponding peak at 1219.5 represents ether group of esters. (R—O—R).

FTIR spectra analysis of *Skimmia* stem crude (Figure 26), aqueous (Figure 27) and *Skimmia* leaves crude (Figure 28) proposed that the stem and leaves extracted nanoparticles presented major absorption bands of infrared radiation at wave numbers 3323.8 cm\(^{-1}\), 3316.0 cm\(^{-1}\), 3315.1 cm\(^{-1}\), 1639.7 cm\(^{-1}\) and 1639.8 cm\(^{-1}\) respectively. By comparing the FTIR spectrum of AuNPs to the FT-IR table values, it was discovered that the infrared radiation absorption band at wave numbers 3323.8 cm\(^{-1}\), 3316.0 cm\(^{-1}\) and 3315.1 cm\(^{-1}\) present in spectra of nanoparticles were found to be in the range of 3200-3550 when compared to the typical infrared absorption frequencies, thus presenting Phenols. The shift in the band discovered that the \(-\text{O—H}\) functional group holding compounds were primarily accountable for reducing the gold (Au) metal. It was also noticed that absorption band at wave numbers 1639.7 cm\(^{-1}\) and 1639.8 cm\(^{-1}\) in spectra of nanoparticles were found to be in the range of 1600-1750 thus presenting esters. Sharp Peaks at 1639.7 cm\(^{-1}\) and 1639.8 cm\(^{-1}\) represent carbonyl group of esters. (C\(\equiv\)O).

**Scanning Electron Microscopic (SEM) study of the gold nanoparticles**

The technique used for the size and shape determination of the gold nanoparticles formed from the roots and shoots, methanolic crude and aqueous extracts of *Viola pilosa* and leaves and stem, methanolic crude and aqueous extracts of *Skimmia laureola* was Scanning Electron Microscopy, formally known as SEM.
Figure 21: FT-IR spectra of *Viola* roots crude extracted gold NPs

Figure 22: FT-IR spectra of *Viola* shoot crude extracted gold NPs
**Figure 23:** FT-IR spectra of *Viola* roots aqueous extracted gold NPs

**Figure 24:** FT-IR spectra of *Viola* shoots aqueous extracted gold NPs
**Figure 25:** FT-IR spectra of *Skimmia* leaves aqueous extracted gold NPs

**Figure 26:** FT-IR spectra of *Skimmia* stem crude extracted gold NPs
Figure 27: FT-IR spectra of *Skimmia* stem aqueous extracted gold NPs

Figure 28: FT-IR spectra of *Skimmia* leaves crude extracted gold NPs
SEM study of the gold nanoparticles of Viola pilosa roots crude extracts

Figure 29 illustrates the results obtained from scanning study of the gold nanoparticles created from the root crude extract of Viola pilosa. The scanning electron microscopic images indicated that the AuNPs were found to be round in shape presenting a normal size of about 20 nm. It was also noticed that a good extent of homogeneity was found between the particles. However, the AuNPs were not clustered together and were distributed separately.

SEM study of the gold nanoparticles of Viola pilosa roots aqueous extracts

SEM technique was used for the determination of morphological characters of the Viola roots aqueous gold nanoparticles (Figure 30). Comprehension of the image results described that the root extract AuNPs were spherical and round in morphology and presented various particle sizes with a value of 25 nm. The nanoparticles were homogenous in nature with a distinctive distribution.

SEM study of the gold nanoparticles of Viola pilosa shoots crude extracts

For determination of the particles shape and size dissemination of the AuNPs produced from the shoot crude extract of Viola pilosa, the samples were subjected to SEM analysis (Figure 31). The results of the image exposed different sizes of the nanoparticles with an average of 20 nm that the newly formed AuNPs were found to be of diverse sizes with a typical size of about 20 nm. The AuNPs were found to be nanospheric and slightly round in shape.

SEM study of the gold nanoparticles of Viola pilosa shoot aqueous extracts

Data regarding the morphology of the shoot aqueous extracted nanoparticles (AuNPs) through a high-magnification scanner is presented in Figure 32. The said image specifies the monodispersed particles presenting an average particle size of 50 nm. A thorough look at the particles revealed a round slightly clustered shape of the stem aqueous extracted AuNPs.
Figure 29: SEM representation of *Viola pilosa* roots crude AuNPs

Figure 30: SEM representation of *Viola pilosa* roots aqueous AuNPs
Figure 31: SEM representation of *Viola pilosa* shoot crude AuNPs

Figure 32: SEM representation of *Viola pilosa* shoots aqueous AuNPs
SEM study of the gold nanoparticles of *Skimmia laureola* stem crude extracts

Figure 33 illustrates the results obtained from scanning examination of the gold nanoparticles created from the stem crude extract of *Skimmia laureola*. The scanning electron microscopic image showed that the AuNPs were found to be round and slightly clustered in shape presenting a regular size of 50 nm. Moreover, it was noticed that a good extent of homogeneity was found between the particles.

SEM study of the gold nanoparticles of *Skimmia laureola* stem aqueous extracts

SEM technique was chosen to define the morphological characters of the *Skimmia* stem aqueous gold nanoparticles (Figure 34). Comprehension of the image results described that the root extract AuNPs were round and cylindrical in morphology and presented various particle sizes with a normal value of 60 nm. The particles were homogenous in nature with an agglomerated distribution.

SEM study of the gold nanoparticles of *Skimmia laureola* leaves crude extracts

For determining the particle shape and size distribution of the AuNPs produced from the leaves crude extract of *Skimmia laureola*, the samples were subjected to SEM analysis (Figure 35). The results of the image exposed different sizes of the nanoparticles with an average of 20 nm that the newly formed AuNPs were of different sizes with a typical size of 24 nm. The AuNPs were found to be in a nanospheric homogenous nature.

SEM study of the gold nanoparticles of *Skimmia laureola* leaves aqueous extracts

Data regarding the morphology of the leaves aqueous extracted nanoparticles (AuNPs) through a high-magnification scanner is presented in Figure 36. The image outcomes specify the monodispersed particles presenting an average particle size of 50 nm. A thorough look at the particles revealed a round spherical shape of the leaves aqueous extracted AuNPs.
Figure 33: SEM representation of *Skimmia laureola* stem crude AuNPs

Figure 34: SEM representation of *Skimmia laureola* stem aqueous AgNPs
Figure 35: SEM representation of *Skimmia laureola* leaves crude AuNPs

Figure 36: SEM representation of *Skimmia laureola* leaves aqueous AuNPs
Biogenic synthesis of silver nanoparticles (AgNPs)

A solution of .1 mM of AgNO\textsubscript{3} was taken for the bioinspired production of silver nanoparticles. The plant extracts were observed optically and were then confirmed by the help of UV-Vis Spectrophotometric technique. There are free electrons in AgNPs that result in rising of SPR or Surface Plasmon Resonance. The UV-Vis Spectrophotometer detects a resonance which is produced by the collective vibration of the silver nanoparticles and light waves and thus is a proof of nanoparticles formation.

Optimization levels for the production of Silver Nano-Particles:

Several changes ranging from concentration of fractions and synthesis to the UV–Vis spectra were adopted during the course of the development of the proper protocols for the production of gold and silver nanoparticles from the crude methanolic and aqueous fractions of Viola pilosa & Skimmia laureola. At the initiation of experiment, the first protocol was designed for the production of silver nano-particles. The optimization of the planned protocols for the production of silver nano-particles from the crude methanolic and aqueous fractions of the roots and shoots of Viola pilosa & Skimmia laureola leaves and stem resulted in either zero peaks or abnormal peaks. Very low concentrations of silver nitrate and plant extract fractions were used for the preliminary protocols designed for the production of crude and aqueous extracted silver NPs. Two methods of stirring and no stirring were adopted. No change in color and no definite peaks were observed for the non-stirring extracts when scanned with Spectrophotometer. The modification in the ratios of silver nitrate, crude methanolic and aqueous fractions of Viola pilosa & Skimmia laureola in stirring protocol resulted in gradual synthesis of the silver nanoparticles. It was clearly detected that an increase in the ratio of the plant extracted fraction and AgNO\textsubscript{3} resulted in denser color of the solutions which was a sign of synthesis of a good amount of nanoparticles. The standard for selecting a healthier ratio was dependent on the shape of the curve on UV–Vis analysis. The ratio with a finer and sharper curve was designated as the optimum peak ratio.
The final optimized extract ratios chosen for advance studies were 1:5 (1ml of AgNO₃ (0.1mM) and 5ml of plant extract) for *Viola* root crude extracts, *Viola* roots aqueous, *Skimmia* stem aqueous and *Skimmia* leaves aqueous extracts, 1:10 (1ml of AgNO₃ (0.1mM) and 10ml of plant extract) for the *Skimmia* stem crude. Similarly, a ratio of 10:1 (10ml of AgNO₃ (0.1mM) and 1ml of plant extract) was observed for the *Viola* shoot crude, *Viola* shoot aqueous and *Skimmia* leaves crude extracts. The results indicated a separate color for each type of nanoparticles synthesized from crude methanolic and aqueous fractions of *Viola pilosa* & *Skimmia laureola*.

**Bioinspired formation of AgNPs of Viola roots and shoots extracts**

The visible change in color and sharper peaks of UV-Vis Spectroscopic analysis specified the formation of silver nanoparticles. The solution for biosynthesis of nanoparticles was made by using 25 ml of .1 Mm AgNO₃ solution and 25 ml (25%) of *Viola pilosa* shoots and roots crude and aqueous extracts. The mixture was incubated, for next 30 minutes at a room temperature with constant shaking, which resulted in the change of colorless extract to a deep brown thick solution which was a strong signal for the production of silver nanoparticles.

**UV-Vis confirmation of Viola roots crude AgNPs**

The UV spectrum shown in Figure 37 characterizes the AgNPs synthesized from the ratio of the 0.1 mM AgNO₃ solution and *Viola pilosa* roots crude extract. The obtained spectrum specified that the highest peak was observed by the combination of 1ml of AgNO₃ solution and 5ml of the plant crude root extract (1:5). Final analysis of the curve for *Viola* root crude revealed an absorbance of 1.487 of the nanoparticles solution at the highest peak of 415.04 nm wavelength. The optimum range for the AgNPs is between 350 nm - 450 nm. The wavelength of 415.04 nm comes within the required range hence confirming the formation of the silver nanoparticles.
Figure 37: UV-Vis confirmation of *Viola* roots crude AgNPs formed by combination of 1ml of AgNO$_3$ solution (0.1mM) and 5ml *Viola* roots crude solution presenting AgNPs peak at 415.04 nm.

Figure 38: UV-Vis confirmation of *Viola* roots aqueous AgNPs formed by combination of 1ml of AgNO$_3$ solution (0.1mM) and 5ml *Viola* roots aqueous solution presenting AgNPs peak at 380.70 nm.
UV-Vis confirmation of *Viola* roots aqueous AgNPs

Figure 38 signifies the AgNPs formed by the ratio of the 0.1 mM AgNO₃ solution and *Viola pilosa* roots aqueous. The spectrum curve indicated that the maximum peak was noticed for 1ml of AgNO₃ solution and 5ml of the *Viola* root aqueous extract (1:5). As per results, the highest sharp peak was observed at 1:5 and was thus used for the further analysis of nanoparticles. The results of spectra analysis for *Viola* root aqueous extracts exposed an absorbance (1.600) of the nanoparticles extract solutions at the highest wavelength of 380.70 nm. The 380.70 nm fits into the wavelength of (350 nm - 450 nm) thus approving the presence of the AgNPs in the extract solution.

UV-Vis confirmation of *Viola* shoots crude AgNPs

The UV spectrum shown in Figure 39 characterizes the AgNPs synthesized from the ratio of the 0.1 mM AgNO₃ solution and *Viola pilosa* shoot crude extract. The obtained spectrum specified that the highest peak was observed by the combination of 10 ml of AgNO₃ solution and 1ml of the plant crude root extract (1:10). Final analysis of the curve for *Viola* shoot crude revealed an absorbance 1.993 of the nanoparticles solution at the highest peak of 400.93 nm wavelength. The optimum range for the AgNPs is between 350 nm - 450 nm. The wavelength of 400.93 nm comes within the required range hence confirming the creation of the silver nanoparticles.

UV-Vis confirmation of *Viola* shoots aqueous AgNPs

Figure 40 signifies the AgNPs formed by the ratio of the 0.1 mM AgNO₃ solution and *Viola pilosa* shoots aqueous extract. The spectrum curve indicated that the highest peak was noticed for 10ml of AgNO₃ solution and 1ml of the *Viola* root aqueous extract (10:1). As per results, the highest sharp peak was observed at 10:1 and was thus used for the further analysis of nanoparticles. The results of spectra analysis for *Viola* shoot aqueous extracts exposed an absorbance (1.994) of the nanoparticles extract solutions at the highest wavelength of 395.66 nm. The 395.66 nm fits into the wavelength of (350 nm – 450 nm) thus approving the presence of the AgNPs in the extract solution.
Figure 39: UV-Vis confirmation of Viola shoots crude AgNPs formed by combination of 10ml of AgNO$_3$ solution (0.1mM) and 1ml Viola shoots crude solution presenting AgNPs peak at 400.93 nm.

Figure 40: UV-Vis confirmation of Viola shoots aqueous AgNPs formed by combination of 10ml of AgNO$_3$ solution (0.1mM) and 1ml Viola shoots aqueous solution presenting AgNPs peak at 395.66 nm.
Stability analysis of AgNPs of *Viola pilosa* roots and shoots

After synthesis of nanoparticles, the study was proceeded to check the stability of AgNPs of *Viola pilosa* roots and shoots by passing them through salt and heat stress. The UV-Vis spectrophotometer was chosen to find out the distinctive effects of heat and salt stress parameters on the silver nanoparticles.

Heat stress stability analysis of *Viola pilosa* roots and shoots AgNPs

The crude and aqueous extracted nanoparticles from roots and shoots of *Viola pilosa* were subjected to different degrees of temperatures. The results showed that the stability of nanoparticles was inversely proportional to the increasing temperatures. The results of Figure 41 shows that the nanoparticles in case of *Viola* root crude, *Viola* root aqueous, *Viola* shoot crude and *Viola* shoot aqueous extracted silver nanoparticles were found highly stable at the temperatures between 25°C and 50°C giving the highest peak at an absorbance level of 2.979. The study proved that rise in temperature reduced the stability of the silver nanoparticles in a temperature ranging from 50°C and 60°C thus lowering the peak of the tested samples. However, the analysis of *Viola* crude and aqueous extracted AgNPs also indicated that the nanoparticles were totally unstable and were damaged at a temperature range of 60°C and 100°C presenting no absorbance through spectrophotometer.

Salt stress stability analysis of *Viola pilosa* roots and shoots AgNPs

Sodium chloride was used as a standard salt to examine the influence of salt stress on the silver nanoparticles of *Viola pilosa* roots and shoots extracts. The experimental protocol started with preparation of three different concentrations of salt (0.1mM, 0.5mM and 1M) which were added separately to the nanoparticles solutions. The results of experiment recommended that an inverse relationship was found between the concentration of Sodium chloride and degree of stability of nanoparticles. An increase in the NaCl concentration resulted in the reduced stability of the AgNPs.
**Figure 41:** UV-Vis spectrum comparison of heat stability of *Viola* shoots and roots AgNPs: (a) *Viola* root crude AgNPs, (b) *Viola* root aqueous AgNPs, (c) *Viola* shoot crude AgNPs, (d) *Viola* shoot aqueous AgNPs.
The highest peak in Figure 42 presents the pure AgNPs solution with no salt in it. The second highest peak represents the decrease in stability when 0.1mM salt solution was added to the nanoparticles. Addition of more salt concentrations (0.5mM and 1M NaCl) resulted in instability of the silver nanoparticles. The UV-spectroscopic absorption and sharper peaks clearly show the negative effects of salt stress on stability of the AgNPs. Moreover, it was also noticed that the molar (1M) concentration of NaCl adversely effected the AgNPs as compared to the milliMolar concentrations (0.1 mM and 0.5 mM). Amongst the tested concentrations, the nanoparticles were found much stable at 0.1 mM, comparatively moderately stable at 0.5 mM and least stable at a salt concentration of 1M.

**UV-Vis confirmation of *Skimmia laureola* stem crude AgNPs**

The UV spectrum shown in Figure 43 characterizes the AgNPs synthesized from the ratio of the 0.1 mM AgNO$_3$ solution and *Skimmia laureola* stem crude extract. The obtained spectrum specified that the highest peak was observed by the combination of 1ml of AgNO$_3$ solution and 5ml of the plant crude root extract (1:5). Final analysis of the curve for *Skimmia* stem crude revealed an absorbance of 2.989 of the nanoparticles solution at the highest peak of 381.99 nm wavelength. The optimum range for the AgNPs is between 350 nm - 450 nm. The wavelength of 381.99 nm comes within the required range hence confirming the creation of the silver nanoparticles.

**UV-Vis confirmation of *Skimmia* stem aqueous AgNPs**

Figure 44 signifies the AgNPs formed by the ratio of the 0.1 mM AgNO$_3$ solution and *Skimmia* stem aqueous extract. The spectrum curve indicated that the uppermost peak was noticed for 1ml of AgNO$_3$ solution and 5ml of the *Skimmia* stem aqueous extract (1:5). As per results, the highest sharp peak was observed at 1:10 and was thus used for the further analysis of nanoparticles. The results of spectra analysis for *Skimmia* stem aqueous extracts exposed an absorbance 2.989 of the nanoparticles extract solutions at the highest wavelength of 395.0 nm. The 395.0 nm fits into the wavelength of (350 nm - 450 nm) thus approving the presence of the AgNPs in the plant extracted solution.
Figure 42: UV-Vis spectrum comparison of salt stress stability of *Viola* shoots and roots AgNPs (a) *Viola* root crude AgNPs, (b) *Viola* root aqueous AgNPs, (c) *Viola* shoot crude AgNPs, (d) *Viola* shoot aqueous AgNPs.
**Figure 43:** UV-Vis confirmation of *Skimmia* stem crude AgNPs formed by combination of 1ml of AgNO₃ solution (0.1mM) and 10ml *Skimmia* stem crude solution presenting AgNPs peak at 381.99 nm.

**Figure 44:** UV-Vis confirmation of *Skimmia* stem aqueous AgNPs formed by combination of 1ml of AgNO₃ solution (0.1mM) and 5ml *Skimmia* stem crude solution presenting AgNPs peak at 397.02 nm.
**UV-Vis confirmation of *Skimmia laureola* leaves crude AgNPs**

The UV spectrum shown in Figure 45 characterizes the AgNPs synthesized from the ratio of the 0.1 mM AgNO$_3$ solution and *Skimmia laureola* leaves crude extract. The obtained spectrum specified that the highest peak was observed by the combination of 10ml of AgNO$_3$ solution and 1ml of the plant crude root extract (10:1). Final analysis of the curve for *Skimmia* leaves crude revealed an absorbance of 2.493 of the nanoparticles solution at the highest peak of 421.36 nm wavelength. The optimum range for the AgNPs is between 350 nm - 450nm. The wavelength of 421.36 nm comes within the required range hence confirming the creation of the silver nanoparticles.

**UV-Vis confirmation of *Skimmia* leaves aqueous AgNPs**

Figure 46 signifies the AgNPs formed by the ratio of the 0.1 mM AgNO$_3$ solution and *Skimmia* leaves aqueous extract. The spectrum curve indicated that the uppermost peak was noticed for 1ml of AgNO$_3$ solution and 5ml of the *Skimmia* leaves aqueous extract (1:5). As per results, the highest sharp peak was observed at 1:5 and was thus used for the further analysis of nanoparticles. The results of spectra analysis for and *Skimmia* leaves aqueous extracts exposed an absorbance (1.981) of the nanoparticles extract solutions at the highest wavelength of 401.59 nm. The 401.59 nm fits into the wavelength of (350 nm - 450 nm) thus approving the presence of the AgNPs in the extract solution.

**Stability analysis**

**Heat stress stability analysis of *Skimmia laureola* stem and leaves AgNPs**

The crude and aqueous extracted nanoparticles from stem and leaves of *Skimmia laureola* were subjected to different degrees of temperatures. The results showed that the stability of nanoparticles was inversely proportional to the increasing temperatures. The results of Figures 47 show that the nanoparticles in case of *Skimmia* stem crude, *Skimmia* stem aqueous, *Skimmia* leaves crude and *Skimmia* leaves aqueous extracted silver nanoparticles were found extremely stable at the temperature ranges of 20°C and 40°C giving the highest peak at an absorbance level of 2.979. The study proved that rise in temperature reduced the stability of the silver nanoparticles in a temperature ranging from 40°C - 60°C thus lowering the peak of the tested
samples. However, the analysis of Skimmia crude and aqueous extracted AgNPs also indicated that the nanoparticles were totally unstable and were damaged at a temperature range of 60°C and 100°C presenting no absorbance through spectrophotometer.

**Salt stress stability analysis of Skimmia laureola stem and leaves AgNPs**

Sodium chloride was used as a standard salt to examine the influence of salt stress on the silver nanoparticles of Skimmia laureola stem and leaves extracts. The experimental protocol started with preparation of three different concentrations of salt (0.1mM, 0.5mM and 1M) which were added separately to the nanoparticles solutions. The results of experiment recommended that an inverse relationship was found between the concentration of Sodium chloride and degree of stability of nanoparticles. An increase in the NaCl concentration resulted in the reduced stability of the AgNPs.

The highest peak in the Figure 48 presents the pure AgNPs solution with no salt in it. The second highest peak represents the decrease in stability when 0.1mM salt solution was added to the nanoparticles. Addition of more salt concentrations (0.5mM and 1M NaCl) resulted in instability of the silver nanoparticles. The UV-spectroscopic absorption and sharper peaks clearly show the negative effects of salt stress on stability of the AgNPs. Moreover, it was also noticed that the molar (1M) concentration of NaCl adversely affected the AgNPs as compared to the milli Molar concentrations (0.1mM and 0.5mM). Amongst the tested concentrations, the nanoparticles were found much stable at 0.1mM, comparatively moderately stable at 0.5mM and least stable at a salt concentration of 1M.

**X-Ray Diffraction (XRD) study of Viola and Skimmia AgNPs extracts:**

The biologically formed silver nanoparticles of the Viola and Skimmia plants extracts were then subjected to XRD technique for the ratification of the crystalline structure. X-ray diffraction is a well-known technique used for the characterization of crystallographic nature, size dimension, and ideal orientation in solid polycrystalline and powdered samples. This technique is always favored for categorization of indefinite crystalline structures. The XRD analysis pattern indicated the crystalline nature of the silver nanoparticles.
Figure 45: UV-Vis confirmation of *Skimmia* leaves crude AgNPs formed by combination of 10ml of AgNO$_3$ solution (0.1mM) and 1ml *Skimmia* leaves crude solution presenting AgNPs peak at 421.36 nm.

Figure 46: UV-Vis confirmation of *Skimmia* leaves aqueous AgNPs formed by combination of 1ml of AgNO$_3$ solution (0.1mM) and 5ml *Skimmia* leaves aqueous solution presenting AgNPs peak at 401.59 nm.
Figure 47: UV-Vis spectrum comparison of heat stability of *Skimmia laureola* stem and leaves AgNPs: (a) *Skimmia* stem crude AgNPs, (b) *Skimmia* stem aqueous AgNPs, (c) *Skimmia* leaves aqueous AgNPs.
Figure 48: UV-Vis spectrum comparison of salt stress stability of Skimmia laureola stem and leaves AgNPs: (a) Skimmia stem crude AgNPs, (b) Skimmia stem aqueous AgNPs, (c) Skimmia leaves crudes AgNPs, (d) Skimmia leaves aqueous AgNPs.
X-Ray Diffraction (XRD) analysis of Viola shoot crude AgNPs extracts:

The demonstrative X-Ray diffraction configuration of the powdered silver nanoparticles of Viola shoot crude is presented in figure 49. XRD study indicated three distinctive deflection peaks within the 2 theta range at angles of 13.39°, 29.68° and 38.32° respectively, acting to be indexed in the diverse planes (110), (301) and (411) of the facets of silver nanoparticles. These planes of silver nanoparticles were in correspondence to those present in database of the Joint Committee on Powder Diffraction Standards, USA (JCPDS no. 00-004-0784), approving the pure centrosymmetric structure of the synthesized nanoparticles. The results of the profile declared that the peak at 411 position was found to be the most dominating diffraction pattern amongst all the other peaks of the analyzed nanoparticles. The full width half maximum determination of the most intense peak 411, by the help of Sherrer equation was chosen for the typical size calculation of the AgNPs, which was appeared to be 19.31 nm. The sharpness of the peak undoubtedly specified that the nanoparticles were present in nano region.

X-Ray Diffraction (XRD) study of Viola shoot aqueous AgNPs extracts:

Figure 50 shows the results of the XRD study carried out for the evaluation of the crystalline configuration of the silver nanoparticles produced from the aqueous extract of Viola shoots. Examination of X-Ray diffraction peaks specified distinctive peaks at two theta values of 27.76°, 29.68°, 32.56° and 37.67° parallel to (203), (212), (220) and (222) surfaces of silver NPs respectively. The index of Bragg’s reflections was done on the source of face centered cubic Ag structure. The dimension of the facets revealed the crystal centrosymmetric nature of the synthesized silver nanoparticles. Determining FWHM for the strongest reflection of (222) on XRD data by using Sherrer equation proposed an average size of 18.50 nm.
Figure 49: RD peaks of *Viola* shoot crude AgNPs

Figure 50: RD peaks of *Viola* shoot aqueous AgNPs
X-Ray Diffraction (XRD) study of *Viola* root crude AgNPs extracts:

The X-Ray diffraction conformation of the powdered silver nanoparticles of *Viola* roots crude is presented in Figure 51. XRD study indicated four distinctive deflection peaks within the 2 theta range at angles of 23.94°, 32.23° and 38.32° respectively, acting to be indexed in the diverse planes (100), (211) and (222) of the facets of silver nanoparticles. These planes of silver nanoparticles were in correspondence to those present in database of the Joint Committee on Powder Diffraction Standards, USA (JCPDS no. 00-004-0784), approving the pure crystal centrosymmetric structure of the synthesized nanoparticles. The results of the profile declared that the peak at 211 position was found to be the most dominating diffraction pattern amongst all the other peaks of the analyzed nanoparticles. The full width half maximum determination of the most intense peak (211), by the help of Sherrer equation was used for the typical size calculation of the synthesized AgNPs, which was appeared to be 3.48 nm. The sharpness of the peak undoubtedly specified that the nanoparticles were present in nano region. The statistical data discovered that no peaks for the XRD patterns were merely due to the crystalline contaminations, which showed that the AgNPs were extremely pure in nature.

X-Ray Diffraction (XRD) study of *Viola* root aqueous AgNPs extracts:

Figure 52 illustrates the consequences of the XRD study carried out for the evaluation of the crystalline configuration of the silver nanoparticles produced from the aqueous extract of *Viola* roots. Examination of X-Ray diffraction peaks specified distinctive peaks at two theta values of 38.32°, 44.39° and 64.84° parallel to (111), (200) and (220) surfaces of silver NPs respectively. The index of Bragg’s reflections was done on the source of face centered cubic Ag structure. The dimension of the facets exposed the crystalline centrosymmetric nature of the synthesized silver nanoparticles. Determining FWHM for the strongest reflection of (111) on XRD data by using Sherrer equation proposed a typical size of 17.93nm. The outcomes also concluded that the sharp Bragg diffraction peaks might be a result of capping agents which are stabilizing the silver nanoparticles.
Figure 51: RD peaks of *Viola* root crude AgNPs

Figure 52: RD peaks of *Viola* shoot aqueous AgNPs
X-Ray Diffraction (XRD) analysis of *Skimmia* stem crude AgNPs extracts:

The demonstrative X-Ray diffraction configuration of the powdered silver nanoparticles of *Skimmia* stem crude is presented in Figure 53. XRD study indicated three distinctive deflection peaks within the 2 theta range at angles of 32.89°, 38.95° and 77.94° respectively, acting to be indexed in the diverse planes (102), (104), and (130) of the facets of silver nanoparticles. These planes of silver nanoparticles were in correspondence to those present in database of the Joint Committee on Powder Diffraction Standards, USA (JCPDS no. 00-004-0784), approving the pure crystallographic centrosymmetric structure of the synthesized nanoparticles. The results of the profile declared that the peak at 104 position was found to be the most dominating diffraction pattern amongst all the other peaks of the analyzed nanoparticles. The full width half maximum determination of the most intense peak (104), by the help of Sherrer equation was used for the typical size calculation of the AgNPs, which was appeared to be 5.56 nm. The sharpness of the peak undoubtedly specified that the nanoparticles were present in nano region.

**X-Ray Diffraction (XRD) study of *Skimmia* stem aqueous AgNPs extracts:**

Figure 54 shows the results of the XRD study carried out for the evaluation of the crystalline configuration of the silver nanoparticles produced from the aqueous extract of *Skimmia* stem. Examination of X-Ray diffraction peaks specified distinctive peaks at two theta values of 17.87°, 38.63°, 44.06° and 64.84° parallel to (111), (107), (110) and (119) surfaces of silver NPs respectively. The index of Bragg’s reflections was done on the source of face centered cubic Ag structure. The dimension of the facets revealed the crystalline centrosymmetric nature of the synthesized silver nanoparticles. Determining FWHM for the strongest reflection of (107) on XRD data by using Sherrer equation proposed an average size of 10.32 nm.
Figure 53: RD peaks of *Skimmia* stem crude AgNPs

Figure 54: RD peaks of *Skimmia* stem aqueous AgNPs
X-Ray Diffraction (XRD) study of *Skimmia* leaves crude AgNPs extracts:

The X-Ray diffraction confirmation of the powdered silver nanoparticles of *Skimmia* leaves crude is presented in Figure 55. XRD study indicated five distinctive deflection peaks within the 2 theta range at angles of 19.62°, 29.58°, 32.19°, 38.33° and 63.35° respectively, acting to be indexed in the diverse planes (111), (222), (310), (400) and (541) of the facets of silver nanoparticles. These planes of silver nanoparticles were in correspondence to those present in database of the Joint Committee on Powder Diffraction Standards, USA (JCPDS no. 00-004-0784), approving the pure crystallographic non-centrosymmetric structure of the nanoparticles. The results of the profile declared that the peak at 400 position was found to be the most dominating diffraction pattern amongst all the other peaks of the analyzed nanoparticles. The full width half maximum determination of the most intense peak (400), by the help of Sherrer equation was used for the typical size calculation of the synthesized AgNPs, which was appeared to be 9.50 nm. The sharpness of the peak undoubtedly specified that the nanoparticles were present in nano region. The statistical information also discovered that no peaks for the XRD configurations were simply due to the crystalline impurities, which showed that the AgNPs were extremely pure in nature.

X-Ray Diffraction (XRD) study of *Skimmia* leaves aqueous AgNPs extracts:

Figure 56 illustrates the consequences of the XRD study carried out for the evaluation of the crystalline configuration of the silver nanoparticles produced from the aqueous extract of *Skimmia* leaves. Examination of X-Ray diffraction peaks specified distinctive peaks at two theta values of 25.53°, 38.63° and 65.48° parallel to (100), (110) and (211) surfaces of silver NPs respectively. The index of Bragg’s reflections was done on the source of face centered cubic Ag structure. The dimension of the facets exposed the crystalline centrosymmetric nature of the synthesized silver nanoparticles. Determining FWHM for the strongest reflection of (110) on XRD data by using Sherrer equation proposed a normal size of 11.51nm. The outcomes also concluded that the sharp Bragg diffraction peaks might be a result of capping agents which are stabilizing the silver nanoparticles.
Figure 55: RD peaks of *Skimmia* leaves crude AgNPs

Figure 56: RD peaks of *Skimmia* leaves aqueous AgNPs
Fourier Transformed Infrared (FT-IR) verification of AgNPs:

The silver nanoparticles (AgNPs) produced by using the crude methanolic and aqueous extracts of *Viola pilosa* and *Skimmia laureola* were subjected to Fourier Transformed Infrared (FTIR) spectroscopic analysis for the confirmation of potential functional groups accountable for reduction of silver (Ag⁺) ions.

Fourier Transformed Infrared (FT-IR) spectroscopic study of *Viola* roots and shoots AgNPs

FTIR spectra of *Viola* roots crude (Figure 57), *Viola* shoots methanolic crude (Figure 58), and *Viola* shoots aqueous extract (Figure 59), proposed that the roots and shoots extracted nanoparticles presented major absorption bands of infrared radiation at wave numbers 3320.0 cm⁻¹, 3316.3 cm⁻¹, 3300.9 cm⁻¹, 1639.8 cm⁻¹, 1639.6 cm⁻¹ and 1219.6 cm⁻¹ respectively. By comparing the FTIR spectra of AgNPs to the FT-IR table values, it was discovered that the infrared radiation absorption band at wave numbers 3320.0 cm⁻¹, 3316.3 cm⁻¹ and 3300.9 cm⁻¹ present in spectrum of nanoparticles were found to be in the range of 3200-3550 when compared to the typical infrared absorption frequencies, thus presenting Phenols. The shift in the band discovered that the –O–H functional group holding compounds were mainly accountable for the reduction of silver (Ag) metal. It was also noticed that absorption band at wave numbers 1639.8 cm⁻¹, 1639.6 cm⁻¹ and 1219.6 cm⁻¹ within range of nanoparticles were found to be in the range of 1600 - 1750 and 1200 - 1300, thus presenting esters. Sharp Peaks at 1639.7 cm⁻¹ and 1639.6 cm⁻¹ represent carbonyl group of esters (C═O) and corresponding peak at 1219.6 cm⁻¹ represents ether group of esters. (R–O–R).

FTIR spectra analysis of *Viola* roots aqueous extract proposed that the roots extracted nanoparticles presented major absorption bands of infrared radiation at wave numbers 3319.2 cm⁻¹, and 1639.6 cm⁻¹ respectively. By comparing the FTIR spectrum of AgNPs to the FT-IR table values, it was discovered that the infrared radiation absorption band at wave numbers 3319.2 cm⁻¹ present in spectra of nanoparticles was found to be in the range of 3200 - 3550 when compared to the typical infrared absorption frequencies, thus presenting Phenols. The shift in the band discovered that the –O–H functional group holding compounds were mainly responsible for the
reduction of silver (Ag) metal. It was also noticed that absorption band at wave numbers 1639.6 cm\(^{-1}\) in spectrum of nanoparticles were found to be in the range of 1600 - 1750 thus presenting esters. Sharp peaks at 1639.6 cm\(^{-1}\) represent carbonyl group of esters. (C═O).

**Fourier Transformed Infrared (FT-IR) spectroscopic study of *Skimmia* stem and leaves AgNPs**

FTIR spectra of *Skimmia* stem aqueous (Figure 61), and *Skimmia* leaves aqueous (Figure 62), extracts proposed that the stem and leaves extracted nanoparticles presented major absorption bands of infrared radiation at wave numbers 3304.8 cm\(^{-1}\), 3316.3 cm\(^{-1}\), 1639.8 cm\(^{-1}\) and 1219.6 cm\(^{-1}\) respectively. By comparing the FTIR spectrum of AgNPs to the FT-IR table values, it was discovered that the infrared radiation absorption band at wave number 3304.8 cm\(^{-1}\) and 3316.3 cm\(^{-1}\) present in spectra of nanoparticles were found to be in the range of 3200-3550 when compared to the typical infrared absorption frequencies, thus presenting Phenols. The shift in the band discovered that the –O–H functional group holding compounds were mainly liable for the reduction of silver (Ag) metal. It was also noticed that absorption band at wave numbers 1639.8 cm\(^{-1}\) and 1219.6 cm\(^{-1}\) in spectrum of nanoparticles were found to be in the range of 1600 - 1750 and 1200 - 1300, thus presenting esters. Sharp Peaks at 1639.8 cm\(^{-1}\) represent carbonyl group of esters. (C═O) and corresponding peak at 1219.6 represents ether group of esters (R–O–R).

FTIR spectra analysis of *Skimmia* stem crude (Figure 63), and *Skimmia* leaves crude (Figure 64), proposed that the stem and leaves extracted nanoparticles presented major absorption bands of infrared radiation at wave numbers 3315.8 cm\(^{-1}\), 3320.0 cm\(^{-1}\) and 1639.8 cm\(^{-1}\) respectively. By comparing the FTIR spectrum of AgNPs to the FT-IR table values, it was discovered that the infrared radiation absorption band at wave numbers 3315.8 cm\(^{-1}\) and 3320.0 cm\(^{-1}\) present in spectrum of nanoparticles were found to be in the range of 3200 - 3550 when compared to the typical infrared absorption frequencies, thus presenting Phenols. The shift in the band discovered that the –O–H functional group holding compounds were mainly responsible for the reduction of silver (Ag) metal. It was also noticed that absorption band at wave number 1639.8 cm\(^{-1}\) was found to be in the range of 1600 - 1750 thus presenting esters. Sharp Peaks at 1639.8 cm\(^{-1}\) represent carbonyl group of esters (C═O).
**Figure 57:** FT-IR spectra of *Viola* roots crude extracted silver NPs

**Figure 58:** FT-IR spectra of *Viola* shoots crude extracted silver NPs
Figure 59: FT-IR spectra of *Viola* shoots aqueous extracted silver NPs

Figure 60. FT-IR spectra of *Viola* roots aqueous extracted silver NPs
Figure 61: FT-IR spectra of *Skimmia* stem aqueous extracted silver NPs

Figure 62: FT-IR spectra of *Skimmia* leaves aqueous extracted silver NPs
Figure 63: FT-IR spectra of *Skimmia* stem crude extracted silver NPs

Figure 64: FT-IR spectra of *Skimmia* leaves crude extracted silver NPs
Scanning Electron Microscopic (SEM) study of the silver nanoparticles

The technique chosen for the size and shape determination of the silver nanoparticles formed from the roots and shoots, methanolic crude and aqueous extracts of *Viola pilosa* and leaves and stem, methanolic and aqueous extracts of *Skimmia laureola* was Scanning Electron Microscopy, formally known as SEM.

**SEM study of the silver nanoparticles of *Viola pilosa* roots crude extracts**

Figure 65 illustrates the results obtained from scanning analysis of the silver nanoparticles produced from the root crude extracts of *Viola pilosa*. The scanning electron microscopic images exposed that the AgNPs were found to be spherical in shape presenting a typical size of less than 20 nm. It was also noticed that a good extent of homogeneity was found between the particles. However the AgNPs were not clustered together and were distributed separately.

**SEM study of the silver nanoparticles of *Viola pilosa* roots aqueous extracts**

SEM technique was done to examine the morphological characters of the *Viola* roots aqueous silver nanoparticles (Figure 66). Comprehension of the image results described that the root extract AgNPs were spherical and round in morphology and presented various particle sizes with an average value of 20 nm. The particles were homogenous in nature with a distinctive distribution.

**SEM study of the silver nanoparticles of *Viola pilosa* shoots crude extracts**

For determining the particle shape and size distribution of the AgNPs produced from the shoot crude extract of *Viola pilosa*, the samples were subjected to SEM analysis (Figure 67). The results of the image exposed different sizes of the nanoparticles with an average of 20 nm that the AuNPs were of diverse sizes with an average of 24 nm. The AgNPs were found to be nanospheric and slightly irregular in shape.
Figure 65: SEM representation of *Viola pilosa* roots crude AgNPs

Figure 66: SEM representation of *Viola pilosa* roots aqueous AgNPs
Figure 67: SEM representation of *Viola pilosa* shoot crude AgNPs

Figure 68: SEM representation of *Viola pilosa* shoots aqueous AgNPs
SEM study of the silver nanoparticles of Viola pilosa shoot aqueous extracts

Data regarding the morphology of the shoot aqueous extracted nanoparticles (AgNPs) through a high-magnification scanning is displayed in Figure 68. The image effects specify the monodispersed particles presenting a particle size of 25 nm. A thorough look at the particles revealed a non-clustered spherical shape of the stem aqueous extracted AgNPs.

SEM study of the silver nanoparticles of Skimmia laureola stem crude extracts

Figure 69 illustrates the results obtained from scanning analysis of the silver nanoparticles produced from the stem crude extracts of Skimmia laureola. The scanning electron microscopic images indicated that the AgNPs were found to be spherical and slightly irregular in shape presenting a regular size of 20 nm. It was also noticed that a good extent of homogeneity was found between the particles. However the AgNPs were tightly clustered together.

SEM study of the silver nanoparticles of Skimmia laureola stem aqueous extracts

SEM technique was carried out to determine the morphological characters of the Skimmia stem aqueous silver nanoparticles (Figure 70). Comprehension of the image results described that the root extract AgNPs were spherical and cylindrical in morphology and presented various particle sizes with an average value of 50 nm. The particles were homogenous in nature with an agglomerated distribution.

SEM study of the silver nanoparticles of Skimmia laureola leaves crude extracts

For the examination of particles shape and size distribution of the AgNPs produced from the leaves crude extract of Skimmia laureola, the samples were subjected to SEM analysis (Figure 71). The results of the image exposed different sizes of the nanoparticles with an average of 80 nm that the AuNPs were of various sizes with an average of 24 nm. The AgNPs were found to be nanospheric clusters having a slightly irregular shape.
Figure 69: SEM representation of *Skimmia laureola* stem crude AgNPs

Figure 70: SEM representation of *Skimmia laureola* stem aqueous AgNPs
Figure 71: SEM representation of *Skimmia laureola* leaves crude AgNPs

Figure 72: SEM representation of *Skimmia laureola* leaves aqueous AgNPs
SEM study of the silver nanoparticles of *Skimmia laureola* leaves aqueous extracts

Data regarding the morphology of the leaves aqueous extracted nanoparticles (AgNPs) through a high-magnification scanning is presented in Figure 72. The image effects specify the monodispersed particles presenting an average particle size of 50 nm. A thorough look at the particles revealed a highly clustered cylindrical shape of the leaves aqueous extracted AgNPs.

**OBJECTIVE 2 and 3:**

Antimicrobial potential of silver nanoparticles and gold nanoparticles (AgNPs and AuNPs) of various solvent extracted roots and shoots fractions of *Viola pilosa*

In the present research *Viola pilosa* and *Skimmia Laureola* were also evaluated for the antimicrobial properties for the confirmation of medicinal value of these plants. For this purpose, six microbial strains were selected and used in the study. The method of disc diffusion was selected to carry out the experiment.

**Antimicrobial potential of gold nanoparticles (AuNPs) of crude methanolic extract of roots of Viola pilosa**

The statistical results regarding the antibacterial potential of the AuNPs of methanolic crude fractions from the roots of *Viola pilosa* is illustrated in Figure 73. Experimental results discovered that in case of *Viola* root crude gold nanoparticles, the highest zone of inhibition (41.66%) was showed by *P. aeruginosa* at a concentration of 18ul disc⁻¹ followed by the same microbe with a value of 37.5% at concentration of 12ul disc⁻¹. The third highest value of inhibition (36.54%) was showed by *Xanthomonas* at a concentration of 18ul disc⁻¹. The minimum inhibition zone (19.28%) was noticed for *K. pnemoniae* at a 6ul disc⁻¹ concentration. The statistical analysis also proposed that *S. aureus, Xanthomonas* and *E. coli* presented good sensitivity to AuNPs, whereas, *K. pnemoniae* and *B. subtilis* exhibited moderate sensitivity to the nanoparticles.
Figure 73: Antibacteril potential, with Standard deviation of gold nanoparticles (AuNPs) of crude methanolic extract of Viola pilosa roots against six bacterial strains by using disc diffusion method.

Figure 74: Antibacteril potential, with Standard deviation of gold nanoparticles (AuNPs) of aqueous extract of Viola pilosa roots against six bacterial strains by using disc diffusion method.
Antibacterial activities of gold nanoparticles (AuNPs) of aqueous extract of roots of *Viola pilosa*

Figure 74 shows the statistical outcomes of the antibacterial activities of the AuNPs of the aqueous extracts from the roots of *Viola pilosa*. Experimental results revealed that in case of *Viola* root aqueous gold nanoparticles, the uppermost zone of inhibition (40.25%) was exposed by *P. aeruginosa* at a concentration of 18ul disc\(^{-1}\) followed by the same microbe with a value of 37.5% and 33.33% at concentrations of 12ul disc\(^{-1}\) and 6ul disc\(^{-1}\) respectively. The lowest zone of inhibition of 18.42% was noticed for *K. pnemoniae*. The statistical results further suggested that *Xanthomonas*, *E.coli*, *B. subtilis*, *P. aeruginosa* and *S. aureus* showed significant inhibition while *K. pnemoniae* showed less sensitivity to the water extracted AuNPs at all the three concentrations under study.

Antibacterial activities of gold nanoparticles (AuNPs) of crude methanolic extract of shoots of *Viola pilosa*

The statistical results regarding the antimicrobial strength of the AuNPs of methanolic crude fraction from the shoots of *Viola pilosa* is illustrated in Figure 75. Experimental results discovered that in case of *Viola* shoot crude gold nanoparticles, the highest zone of inhibition (41.66%) was showed by *P. aeruginosa* at a concentration of 18µl disc\(^{-1}\) followed by the same microbe with a value of 37.5% at a concentration of 12 µl disc\(^{-1}\). The third highest value of sensitivity (35.48%) was shown by *Xanthomonas*. While the minimum inhibition zone (19.28%) was noticed for *K. pnemoniae* at 6µl disc\(^{-1}\) concentration. The statistical analysis also proposed that *Xanthomonas*, *E. coli* and *S. aureus* presented maximum sensitivity to AuNPs, whereas, *K.pnemoniae* and *B. subtilis* exhibited moderate sensitivity to the nanoparticles.
Antibacterial activities of gold nanoparticles (AuNPs) of aqueous extract of shoots of Viola pilosa

Figure 76 shows the statistical outcomes of the antibacterial activities of the AuNPs of the aqueous extracts from the roots of Viola pilosa. Experimental results revealed that in case of Viola root aqueous gold nanoparticles, the maximum zone of inhibition (45.83%) was exhibited by P. aeruginosa at a concentration of 18µl disc\(^{-1}\) followed by the same microbe with a value of 41.66% and 37.5% at concentrations of 12µl disc\(^{-1}\) and 6µl disc\(^{-1}\) respectively. The lowest zone of inhibition of 17.45% was noticed for K. pnemoniae. The statistical results further suggested that E.coli, Xanthomonas and S.aureus showed significant inhibition while K.pnemoniae and B. subtilis showed less sensitivity to the water extracted AuNPs at all the three concentrations under study.

Antibacterial activities of gold nanoparticles (AuNPs) of crude methanolic extract of stem of Skimmia Laureola

The statistical consequences about the antibacterial potential of the AuNPs prepared from the methanolic crude samples from the stem of Skimmia Laureola is demonstrated in Figure 77. Experimental outcomes revealed that in case of Skimmia stem crude gold nanoparticles, the topmost zone of inhibition (48.66%) was displayed by P. aeruginosa at a concentration of 18µl disc\(^{-1}\) followed by the same microorganism with a value of 37.5% at a concentration of 12µl disc\(^{-1}\). The third most value of inhibition 35.48% was presented by Xanthomonas at a concentration of 18µl disc\(^{-1}\). However, the lowest inhibitory zone (23.68%) was noticed for K. pnemoniae and B. subtilis at a 6µl disc\(^{-1}\) concentration. The statistical study also recommended that S.aureus and E. coli and presented extreme sensitivity to AuNPs, but K. pnemoniae and B. subtilis showed moderate sensitivity to the gold nanoparticles.

Antibacterial activities of gold nanoparticles (AuNPs) of aqueous extract of stem of Skimmia Laureola

Figure 78 illustrates the statistical records of the antimicrobial potential of the AuNPs of the water extracted samples from the stem of Skimmia Laureola. The results of the study discovered that in case of Skimmia Laureola stem aqueous gold nanoparticles, the maximum value of inhibition (49.6%) was revealed by P.aeruginosa, at a concentration of 18µl disc\(^{-1}\) followed by the same microorganism.
with a value of 38.87% and 37.5% at concentrations of 12µl disc⁻¹ and 6µl disc⁻¹. While the third highest zone inhibitory value (32.25%) was noticed for Xanthomonas and S.aureus at 18µl disc⁻¹ concentration. The lowest inhibitory zone value of 18.42% was noticed for K. pnemoniae. The statistical results further suggested that E. coli and B. subtilis showed good response against the AgNPs, while K. pnemoniae showed less sensitivity to the water extracted AuNPs at all the three concentrations under study.

**Antibacterial activities of gold nanoparticles (AuNPs) of crude methanolic extract of leaves of Skimmia Laureola**

The statistical consequences about the antibacterial potential of the AuNPs prepared from the methanolic crude samples from the leaves of Skimmia Laureola is demonstrated in Figure 79. Experimental outcomes revealed that in case of Skimmia leaves crude gold nanoparticles, the topmost zone of inhibition (44.6%) was displayed by P. aeruginosa at a concentration of 18µl disc⁻¹ followed by the same microorganism with a value of 37.5 % and 34.7 % at concentrations of 12µl disc⁻¹ and 6µl disc⁻¹ respectively. The fourth highest value of inhibition 33.32 % was presented by S.aureus at a concentration of 18µl disc⁻¹. However, the lowest inhibitory zone (25.8%) was noticed for Xanthomonas at a 6µl disc⁻¹ concentration. The statistical study also recommended that E. coli presented extreme sensitivity to AuNPs, but K. pnemoniae showed moderate sensitivity to the gold nanoparticles.

**Antibacterial activities of gold nanoparticles (AuNPs) of aqueous extract of leaves of Skimmia Laureola**

Figure 80 illustrates the statistical records of the antimicrobial activities of the AuNPs of the water extracts of the leaves of Skimmia Laureola. The results of the research discovered that in case of Skimmia Laureola leaves aqueous gold nanoparticles, the maximum value of inhibition (39.87%) was revealed by P. aeruginosa, at a concentration of 18µl disc⁻¹ followed by the same microorganism with a value of 37.5% and 39.33% at concentrations of 12µl disc⁻¹ and 6µl disc⁻¹. While the fourth highest inhibitory zone value (32.25%) was noticed for S. aureus at 18µl disc⁻¹ concentration. The lowest inhibitory zone value of 16.82% was noticed for K. pnemoniae. The statistical results further suggested that E. coli and Xanthomonas showed good response against the AuNPs, while K. pnemoniae and B. subtilis showed less sensitivity to the water extracted AuNPs at all the three concentrations under study.
Figure 75: Antibacterial potential, with Standard deviation of gold nanoparticles (AuNPs) of crude methanolic extract of Viola pilosa shoots against six bacterial strains by using disc diffusion method.

Figure 76: Antibacterial potential, with Standard deviation of gold nanoparticles (AuNPs) of aqueous extract of Viola pilosa shoots against six bacterial strains by using disc diffusion method.
**Figure 77:** Antibacterial potential, with Standard deviation of gold nanoparticles (AuNPs) of crude extracts of *Skimmia Laureola* stem against bacterial strains by using disc diffusion method.

**Figure 78:** Antibacterial potential, with Standard deviation of gold nanoparticles (AuNPs) of water extracts of *Skimmia Laureola* stem against bacterial strains by using disc diffusion method.
Figure 79: Antibacterial potential, with Standard deviation of gold nanoparticles (AuNPs) of crude extracts of *Skimmia Laureola* leaves against bacterial strains by using disc diffusion method.

Figure 80: Antibacterial potential, with Standard deviation of gold nanoparticles (AuNPs) of aqueous extracts of *Skimmia Laureola* leaves against bacterial strains by using disc diffusion method.
Antibacterial activities of silver nanoparticles

Antibacterial activities of silver nanoparticles (AgNPs) of crude methanolic extract of roots of *Viola pilosa*

The statistical results regarding the antimicrobial potentials of the AgNPs synthesized from the crude of the roots of *Viola pilosa* is illustrated in Figure 81. Experimental results discovered that in case of *Viola* root crude silver nanoparticles, the highest zone of inhibition (90.25%) was showed by *P. aeruginosa* at a concentration of 18µl disc\(^{-1}\) followed by the same microbe with a value of 80.33% and 81.91% at concentrations of 12µl disc\(^{-1}\) and 6µl disc\(^{-1}\) respectively. While the minimum inhibition zone (26.87%) was noticed for *Xanthomonas* at a 6µl disc\(^{-1}\) concentration. The statistical analysis also proposed that *P. aeruginosa, B. subtilis* and *E. coli* presented maximum sensitivity to AgNPs, whereas, *Xanthomonas, K. pnemoniae* and *S.aureus* exhibited moderate sensitivity to the nanoparticles.

Antibacterial activities of silver nanoparticles (AgNPs) of aqueous fraction of roots of *Viola pilosa*

Figure 82 shows the statistical outcomes of the antibacterial activities of the AgNPs of the aqueous extract from the roots of *Viola pilosa*. Experimental results revealed that in case of *Viola* root aqueous silver nanoparticles, the maximum zone of inhibition (35.8%) was indicated by *Xanthomonas* at a concentration of 18µl disc\(^{-1}\) followed by the same microbe with a value of 31.16% at a concentration of 12µl disc\(^{-1}\). While the third highest inhibition zone value (30.09%) was noticed for *E. coli* at 18µl disc\(^{-1}\) concentration. The statistical results further suggested that *P. aeruginosa, S. aureus, B. subtilis* and *K. pnemoniae* were proved to be fully resistant to the aqueous extracted AgNPs at all three concentrations under study.
Figure 81: Antibacterial potential, with Standard Deviation of silver nanoparticles (AgNPs) of crude methanolic extract of *Viola pilosa* roots against six bacterial strains by using disc diffusion method.

Figure 82: Antibacterial potential, with Standard Deviation of silver nanoparticles (AgNPs) of aqueous extract of *Viola pilosa* roots against six bacterial strains by using disc diffusion method.
Antibacterial activities of silver nanoparticles (AgNPs) of crude methanolic extract of shoots of Viola pilosa

The statistical results regarding the antimicrobial potentials of the AgNPs of crude extracts from the shoots of Viola pilosa is illustrated in Figure 83. Experimental results discovered that in case of Viola shoot crude silver nanoparticles, the highest zone of inhibition (45.83%) was showed by P. aeruginosa at a concentration of 18µl disc\(^{-1}\) followed by the same microbe with a value of 41.66% and 37.5% at concentrations of 12µl disc\(^{-1}\) and 6µl disc\(^{-1}\) respectively. While the minimum inhibition zone (20.15%) was noticed for K. pneumoniae at a 6µl disc\(^{-1}\) concentration. The statistical analysis also proposed that Xanthomonas, S.aureus and E.coli presented maximum sensitivity to AgNPs, whereas, K. pneumoniae and B. subtilis exhibited moderate sensitivity to the nanoparticles.

Antibacterial activities of silver nanoparticles (AgNPs) of aqueous extract of shoots of Viola pilosa

Figure 84 shows the statistical outcomes of the antibacterial activities of the AgNPs of the aqueous extracts from the shoots of Viola pilosa. Experimental values revealed that in case of Viola shoots aqueous silver nanoparticles, the highest zone of inhibition (41.66%) was showed by P. aeruginosa at a concentration of 18µl disc\(^{-1}\) followed by Xanthomonas with a value of 37.61% at a concentration of 18µl disc\(^{-1}\). While the lowest inhibition zone (21.05%) was noticed for B. subtilis at a 6µl disc\(^{-1}\) concentration. The statistical results further suggested that S. aureus and E. coli showed good sensitivity while B. subtilis and K. pneumoniae presented moderate sensitivity to the water extracted AgNPs at all the three concentrations under study.

Antibacterial activities of silver nanoparticles (AgNPs) of crude methanolic extract of stem of Skimmia Laureola

The statistical consequences about the antibacterial potential of the AgNPs prepared from the methanolic crude samples from the stem of Skimmia Laureola is demonstrated in Figure 85. Experimental outcomes revealed that in case of Skimmia stem crude silver nanoparticles, the topmost zone of inhibition (91.66 %) was displayed by P. aeruginosa at a concentration of 18µl disc\(^{-1}\) followed by the same
microorganism with a value of 88.87% and 86.03% at concentrations of 12µl disc\(^{-1}\) and 6µl disc\(^{-1}\) respectively. Whereas, the lowest inhibition of (31.57%) was noticed for *K. pneumoniae* at a 6µl disc\(^{-1}\) concentration. The statistical study also recommended that *E. coli*, *Xanthomonas* and *S. aureus* presented extreme sensitivity to AgNPs, but *K. pneumoniae* presented moderate sensitivity to the silver nanoparticles.

**Antibacterial activities of silver nanoparticles (AgNPs) of aqueous extracted fraction of stem of *Skimmia Laureola***

Figure 86 illustrates the statistical data of the antibacterial strength of the AgNPs of the water extracted samples from the stem of *Skimmia Laureola*. The results of the study discovered that in case of *Skimmia Laureola* stem aqueous silver nanoparticles, the maximum value of inhibition (41.66%) was revealed by *P. aeruginosa*, at a concentration of 18µl disc\(^{-1}\) followed by the same microorganism with a value of 37.5% and 33.33% at concentrations of 12µl disc\(^{-1}\) and 6µl disc\(^{-1}\). While the third highest zone inhibitory value (35.48%) was noticed for *E. coli* at 18µl disc\(^{-1}\) concentration. The statistical results further suggested that *B. subtilis* and *Xanthomonas* showed good response against the AgNPs. On the other hand, *S. aureus* and *K. pneumoniae* showed complete resistance to the water extracted AgNPs at all the three concentrations under study.

**Antibacterial activities of silver nanoparticles (AgNPs) of crude methanolic extract of leaves of *Skimmia Laureola***

The statistical consequences about the antibacterial potential of the AgNPs prepared from the methanolic crude samples from the leaves of *Skimmia Laureola* is demonstrated in Figure 87. Experimental outcomes revealed that in case of *Skimmia* stem crude silver nanoparticles, the highest zone of inhibition (52.75%) was displayed by *P. aeruginosa* at a concentration of 18µl disc\(^{-1}\) followed by the same microorganism with a value of 45.83% at a concentration of 12µl disc\(^{-1}\). The third highest value of inhibition of 38.7% was presented by *Xanthomonas* at a concentration of 18µl disc\(^{-1}\). However, the lowest inhibitory zone of 21.05% was noticed for *K. pneumoniae* and *B. subtilis* at 6µl disc\(^{-1}\) concentration. The statistical study also recommended that *E. coli*, and *S. aureus* presented extreme sensitivity to
AgNPs. However, *B. subtilis* and *K. pnemoniae* showed moderate sensitivity to the silver nanoparticles.

**Antibacterial activities of silver nanoparticles (AgNPs) of aqueous extracted fraction of leaves of Skimmia Laureola**

Figure 88 illustrates the statistical data of the antibacterial activity of the AgNPs of the water extracted samples from the leaves of *Skimmia Laureola*. The results discovered that in case of *Skimmia Laureola* leaves aqueous silver nanoparticles, the maximum value of inhibition (40.25%) was revealed by *P. aeruginosa* at a concentration of 18µl disc\(^{-1}\) followed by the same microorganism with a value of 36.03% and 33.33% at concentrations of 12µl disc\(^{-1}\) and 6µl disc\(^{-1}\). While the third highest inhibition of value (33.32%) was noticed for *Xanthomonas* at 18µl disc\(^{-1}\) concentration. The statistical results further suggested that *B. subtilis* and *E. coli* showed good response against the AgNPs. On the other hand *S.aureus* and *K. pnemoniae* showed complete resistance to the water extracted AgNPs at all the three concentrations under study.

**Antibacterial activity of Plant Extracts**

**Antibacterial poteential of Viola pilosa root fractions against P. aeruginosa**

The results of Figure 89 illustrate the antibacterial activity of *Viola pilosa* root extracts against *P. aeruginosa*. The data attained from disc diffusion assay exposed that different solvent extracted samples measured varying degree of growth inhibition at all the tested concentrations. The results revealed that growth reduction of the tested microbe was dose dependent. Maximum growth inhibition of 75.41% was noted by ethyl acetate extracts at 2 mg disc\(^{-1}\) concentration followed by butanol (74.04%) at the similar concentration. Similarly, the lowest activity (34.25% ZI) against the same microbe was noticed for aqueous extracts at 0.5 mg disc\(^{-1}\) when matched to other samples and controls.
**Figure 83:** Antibacterial potential, with Standard Deviation of silver nanoparticles (AgNPs) of crude methanolic extract of *Viola pilosa* shoots against six bacterial strains by using disc diffusion method

**Figure 84:** Antibacterial potential, with Standard Deviation of silver nanoparticles (AgNPs) of aqueous extract of *Viola pilosa* shoots against six bacterial strains by using disc diffusion method
Figure 85: Antibacterial potential, with Standard deviation of silver nanoparticles (AgNPs) of crude extracts of *Skimmia Laureola* stem against bacterial strains by using disc diffusion method.

Figure 86: Antibacterial potential, with Standard deviation of silver nanoparticles (AgNPs) of aqueous extracts of *Skimmia Laureola* stem against bacterial strains by using disc diffusion method.
**Figure 87:** Antibacterial potential, with Standard deviation of silver nanoparticles (AgNPs) of crude extracts of *Skimmia Laureola* leaves against bacterial strains by using disc diffusion method.

**Figure 88:** Antibacterial potential, with Standard deviation of silver nanoparticles (AgNPs) of aqueous extracts of *Skimmia Laureola* leaves against bacterial strains by using disc diffusion method.
Figure 89: Antibacterial potential with standard deviation, of five different solvents extracted samples of roots of *Viola pilosa* against *P. aeruginosa*.

Figure 90: Antibacterial potential with standard deviation, of five different solvents extracted samples of roots of *Viola pilosa* against *S. aureus*. 
**Antibacterial activity of Viola pilosa roots extracts against S. aureus**

The activities of different plant extracts fractions of Viola pilosa against S. aureus is shown in Figure 90. Analysis of the data revealed that butanol extract fraction was proficient in controlling the growth of the tested bacterium by 41.19% at the highest concentration of 2 mg disc$^{-1}$ followed by the hexane extract fraction (40.83% ZI) at the same concentration. The data also proposed that aqueous extracted samples from the tested plant was unable to reduce the activity of the said microbe at any concentration used with 0% ZI. Hexane, crude and ethyl acetate extract fractions also reduced the growth process of S. aureus to varying degree.

**Antibacterial activity of Viola pilosa roots extracts against B. subtilis**

Data shown in Figure 91 indicated that different solvent extracted samples reduced the growth of B. subtilis and this growth reduction was dose dependent. The highest growth inhibition (52.60%) was calculated for butanol at 2 mg disc$^{-1}$ followed by 45.6% ZI noted by the same fraction at 1 mg disc$^{-1}$. The records further proposed that the other tested plant fractions also reduced the growth of the same microbe measuring different zones of inhibition at all concentration. It is also evident from the result that lowest antimicrobial strength of 20.44% was revealed by aqueous extracted fraction at 0.5 mg disc$^{-1}$.

**Antibacterial strength of Viola pilosa roots extracts against E. coli**

The outcomes specified that E. coli was also susceptible to different solvent fractions of the tested plant at all concentrations. Maximum growth inhibition of 41.19% was calculated for ethyl acetate extract at the uppermost concentration of 2 mg disc$^{-1}$ followed by 39.41% each of the same extract and crude methanolic at 1 mg disc$^{-1}$. Minimum activity of 25.06% was noted for the same microbe by aqueous extract samples at the lowermost concentration of 0.5 mg disc$^{-1}$ (Figure 92).
Figure 91: Antibacterial potential with standard deviation, of five different solvents extracted samples of roots of *Viola pilosa* against *B. subtilis*.

Figure 92: Antibacterial potential with standard deviation, of five different solvents extracted samples of roots of *Viola pilosa* against *E. coli*. 
Antibacterial strength of *Viola pilosa* roots extracted fractions against *Xanthomonas campestris*

The experimental outcomes revealed that the development of *X. campestris* was inhibited by various fraction at all the concentrations (Figure 93). Maximum activity was noticed for butanol- extracted fraction (62% ZI) at 2 mg disc$^{-1}$ followed by crude extract (55.9% ZI) of the same fraction at 1 mg disc$^{-1}$. The results also indicated that aqueous extract was less efficient in controlling the activity of the same microbe at 0.5 mg disc$^{-1}$ presenting 27.58% ZI.

**Antibacterial activity of Viola pilosa roots extracts against K. pneumoniae**

The antibacterial potential of solvent extract fractions from the roots of *Viola pilosa* against *K. pneumoniae* indicated that the tested microbe was absolutely resistant to all the fractions at all the three concentrations measuring 0% ZI.

**Antibacterial strength of Viola pilosa shoots extracts against K. pneumonia**

The antibacterial potential of solvent extract fractions from the shoots of *Viola pilosa* against *K. pneumonia* is presented in Fig (94). The tested microbe presented high zone of inhibition to ethyl acetate, n-hexane, butanol and crude fractions. The maximum inhibitory zone of 45.89% was given by butanol extract at a concentration of 2 mg disc$^{-1}$ and the lowermost by n-hexane extracted samples 22.2% at a concentration of 0.5 mg disc$^{-1}$. On the other hand, the aqueous extract fraction was unable to show any activity when compared with controls and other extracts.

**Antibacterial activity of Viola pilosa shoots extracts against P. aeruginosa.**

Figure 95 illuminates the probable antibacterial strength of *Viola pilosa* shoots extracted fraction against *P. aeruginosa*. The fallouts showed that the different fractions demonstrated variable grades of antimicrobial activity on the microorganism tested. The data revealed that the reduction in growth of the microbe under study relied totally on dose. Amongst all solvent fractions, the highest zone of inhibition of 53.45% was measured by ethyl acetate extracted sample at 2 mg disc$^{-1}$ concentration by n-hexane (51.37%) at the equivalent concentration. On the other hand, zero activity was exposed by aqueous extracted samples for the said microbe when compared with controls.
Figure 93: Antibacterial potential with standard deviation, of five different solvents extracted samples of roots of *Viola pilosa* against *X. campestris*.

Figure 94: Antibacterial potential with standard deviation, of five different solvents extracted samples of shoots of *Viola pilosa* against *K. pneumoniae*. 
Figure 95: Antibacterial potential with standard deviation, of five different solvents extracted samples of shoots of *Viola pilosa* against *P. aeruginosa*.

Figure 96: Antibacterial potential with standard deviation, of five different solvents extracted samples of shoots of *Viola pilosa* against *S. aureus*. 
Antibacterial activity of *Viola pilosa* shoots extracts against *S. aureus*.

The antimicrobial activity of solvent extracts of *Viola pilosa* against *S. aureus* is illustrated in Figure 96. The results obtained in the study specified that ethyl acetate extract was found more sensitive to inhibit the growth of the tested microbe by 45.12% at the maximum concentration of 2 mg disc$^{-1}$ followed by n-hexane extract (41.38% ZI) at the related concentration. Our data also proposed that the water and butanol extracted fractions from the tested shoots did not control the activity of the same bacterium at all the concentrations used, giving 0% ZI. Fraction of methanolic crude also exposed decent inhibiting activity against *S. aureus*.

Antibacterial strength of *Viola pilosa* shoots extracts against *Bacillus subtilis*.

The data illustrated that the Crude methanolic fraction and other solvent extracted fractions were effective in inhibiting the growth of *Bacillus subtilis* excluding aqueous fraction that was unable to express activity at all three concentration (Figure 97). The topmost inhibition of 35.94% against *Bacillus subtilis* was recorded for n-hexane at the maximum level of 2 mg discs$^{-1}$ followed by ethyl acetate with a zone of inhibition of 32.73% at the similar concentration. Methanol and butanol indicated good amount of activity against *Bacillus subtilis* however, no activity was recorded for aqueous extracted fraction at all three concentrations.

Antibacterial activity of *Viola pilosa* shoots extracts against *Escherichia coli*

*Escherichia coli* exposed maximum susceptibility to n-hexane fractions with maximum growth inhibition activity of 45.12% at the higher concentration of 2 mg disc$^{-1}$ followed by 38.67% and 32.96% zone of inhibition at 1 and 0.5 mg discs$^{-1}$ respectively when matched to other extracts and controls. The records showed that *Escherichia coli* also demonstrated adequate antibacterial activity for ethyl acetate and crude fractions i.e., 37.25% and 36.54% ZI at 2 mg discs$^{-1}$ respectively. These fractions were also found susceptible at 1 and 0.5 mg discs$^{-1}$ for the tested microbe when compared with other fractions and controls. The results furthermore revealed that no activities were recorded for butanol and water extracts against *E. coli* (Figure 98).
Figure 97: Antibacterial potential with standard deviation, of five different solvents extracted samples of shoots of *Viola pilosa* against *Bacillus subtilis*.

Figure 98: Antibacterial potential with standard deviation, of five different solvents extracted samples of shoots of *Viola pilosa* against *E. coli*.
Antibacterial potential of *Viola pilosa* shoots fractions against *Xanthomonas campestris*

The antibacterial strength of solvent extracts from the shoots of *Viola pilosa* against *Xanthomonas campestris* is illustrated in Fig (99). *Xanthomonas campestris* showed resistance for aqueous fraction presenting zero activity at all the concentrations. The topmost value of inhibition was exposed by butanol fraction (48.38% ZI) at concentration of 2 mg disc$^{-1}$ followed by ethyl acetate extracted fraction with a zone inhibition of (41.93%) at 2 mg disc$^{-1}$. The research outcomes also discovered that hexane and crude fractions possessed substantial amount of activity of 39.77% and 33.32 % ZI at the concentration of 2 mg disc$^{-1}$ for *Xanthomonas campestris* when matched to controls.

Antibacterial activity of *Skimmia laureola* stem extracts against *P. aeruginosa*

The results of Figure 100 indicates the antibacterial activity of *Skimmia laureola* stem extracts against *P. aeruginosa*. The statistical data exposed that solvent extracted samples presented different degree of growth inhibition at the three concentrations under study. The results revealed that reduction in growth of the tested microbial organism was dose dependent. Maximum inhibition of 72.20% was noted for ethyl acetate fractioned samples at concentration of 2 mg disc$^{-1}$ followed by hexane (61.75%) at the same concentration. Similarly, minimum activity (39.54% ZI) against the said microbe was measured by butanol extract at 1 mg disc$^{-1}$ as compared to remaining samples and control. The results also revealed zero activity for water extract against *P. aeruginosa*.

Antibacterial strength of *Skimmia Laureola* stem extracts against *B. subtilis*

Data shown in Figure 101 specified that fractions obtained rom different solvent systems reduced the growth of *B. subtilis* and this growth reduction was dose dependent. The highest growth inhibition (49.10%) was recorded for ethyl acetate at 2 mg disc$^{-1}$ followed by 45.15% ZI noted by the same fraction at 1 mg disc$^{-1}$. The outcomes also exposed that the other tested fractions were also found effective in inhibiting the growth of the said microbe measuring different zones of inhibition at all concentration. It is also evident from the results that lowest activity of 24.10% was noticed for butanol extracted fraction at 0.5 mg disc$^{-1}$. The aqueous extract was incapable to show activity against *B. subtilis*. 120
Figure 99: Antibacterial potential with standard deviation, of five different solvents extracted samples of shoots of Viola pilosa against Xanthomonas campestris.

Figure 100: Antibacterial potential with standard deviation, of five different solvents extracted samples of stem of Skimmia Laureola against P. aeruginosa.
Figure 101: Antibacterial potential with standard deviation, of five different solvents extracted samples of stem of *Skimmia Laureola* against *B. subtilis*.

Figure 102: Antibacterial potential with standard deviation, of five different solvents extracted samples of stem of *Skimmia Laureola* against *S. aureus*
Antibacterial activity of *Skimmia Laureola* stem extracts against *S. aureus*

The antimicrobial strength of different plant extracts of *Skimmia Laureola* against *S. aureus* is shown in Figure 102. Analysis of the data revealed that ethyl acetate extract was proficient to control the growth of the tested bacterium by 47.83% at the highest concentration of 2 mg disc\(^{-1}\) followed by hexane extracts (45.16% ZI) at the same concentration. The data recommended that aqueous and butanol extracted samples from the tested fractions did not reduce the activity of the same microbe at any concentration used measuring 0% ZI. Hexane, crude and ethyl acetate extracts also reduced the growth of *S. aureus* to varying degree.

**Antibacterial activity of *Skimmia Laureola* stem extracts against *Xanthomonas campestris***

The outcomes also revealed reduction in growth of *X. campestris* by solvent extracted fraction at all three concentrations (Figure 103). Uppermost value of inhibition was recorded for ethanol extracted fraction (52.67% ZI) at 2mg disc\(^{-1}\) followed by hexane extract (46.74% ZI) at the equivalent concentration of 2mg disc\(^{-1}\). The results further indicated that aqueous extract fractions were unable to control the activity of the same microorganism at any concentration.

**Antibacterial activity of *Skimmia Laureola* stem extracts against *E. coli***

The results indicated that *E. coli* was also susceptible to different solvent fractions of the tested plant at all concentration. Maximum inhibition value of 46.74% was recorded for ethyl acetate fraction at the uppermost concentration of 2 mg disc\(^{-1}\) followed by 44.61% of the hexane extract at the equal concentration of 2 mg disc\(^{-1}\). No activity was noted for the same microbe by aqueous and butanol extracted samples at all the given concentrations (Figure 104).
Figure 103: Antibacterial potential with standard deviation, of five different solvents extracted samples of stem of *Skimmia Laureola* against *X. campestris*.

Figure 104: Antibacterial potential with standard deviation, of five different solvents extracted samples of stem of *Skimmia Laureola* against *E. coli*.
Antibacterial activity of *Skimmia Laureola* stem fractions against *K. pneumoniae*

The antibacterial strength of solvent fractions from the stem of *Skimmia Laureola* against *K. pneumoniae* directed that the test microorganism was completely susceptible to all the solvent fractions except aqueous extract which was incapable to show any activity at all three test concentrations. Maximum reduction in growth of 50% was noticed for ethyl acetate at a concentration of 2 mg disc$^{-1}$ followed by hexane with 47.78% at the equivalent concentration of 2 mg disc$^{-1}$.

**Antibacterial activity of Skimmia Laureola leaves extracts against Bacillus subtilis**

The data illustrated that the Crude methanolic fraction and other solvent extracted fractions reduced the growth of *Bacillus subtilis* excluding aqueous extract that was unable to show result at all three concentration (Figure 106). The uppermost value of inhibition of 57% against *Bacillus subtilis* was observed for ethyl acetate at the maximum concentration of 2 mg discs$^{-1}$ followed by 50.86% of inhibition by the same fraction at a concentration of 1 mg discs$^{-1}$. Methanol, hexane and n-butanol also showed reasonable activity against *Bacillus subtilis* while no inhibition was observed for aqueous fraction at any concentration.

**Antibacterial activity of Skimmia Laureola leaves extracts against K. pneumonia**

The antibacterial strength of solvent fractions from the leaves of *Skimmia Laureola* against *K. pneumonia* is shown in Figure 107. The tested microbe presented high zone of inhibition to n-hexane, ethyl acetate, methanol and butanol extracted fractions. The maximum inhibitory zone of 58.76% was detected for ethyl acetate extract at a concentration of 2 mg disc$^{-1}$ and the lowermost by butanol extracted samples 27.60% at a concentration of 0.5 mg disc$^{-1}$. On the other hand, the aqueous fraction failed to show activity when compared with controls and other extracts.
Figure 105: Antibacterial potential with standard deviation, of five different solvents extracted samples of stem of *Skimmia Laureola* against *K. pneumoniae*.

Figure 106: Antibacterial potential with standard deviation, of five different solvents extracted samples of leaves of *Skimmia Laureola* against *Bacillus subtilis*. 
**Figure 107:** Antibacterial potential with standard deviation, of five different solvents extracted samples of leaves of *Skimmia Laureola* against *K. pneumonia*

**Figure 108:** Antibacterial potential with standard deviation, of five different solvents extracted samples of leaves of *Skimmia Laureola* against *P. aeroginosa*
Antibacterial activity of *Skimmia Laureola* leaves extracts against *P. aeruginosa*

Figure 108 demonstrates the probable antibacterial strength of *Skimmia Laureola* leaves extracts against *P. aeruginosa*. The outcomes disclosed that different fractions demonstrated variable degree of antibacterial strength to the tested microorganism. The data revealed that the reduction in growth was totally dose dependent. Among different extracts, the highest zone of inhibition of 86.79% was measured by ethyl acetate extracted fraction at 2 mg disc⁻¹ concentration followed by n-hexane (86.08%) at the similar concentration. However, no activity was shown by aqueous fractions against the same microbe when matched to controls.

Antibacterial activity of *Skimmia Laureola* leaves extracts against *Escherichia coli*

*Escherichia coli* was found extremely susceptible to the fraction of ethyl acetate presenting growth inhibition of 60.19% at the higher concentration of 2 mg disc⁻¹ followed by 53.32% and 46.90% zone of inhibition at 1 and 0.5 mg discs⁻¹ respectively when matched to other extracts and control. The outcomes specified that *Escherichia coli* also exhibited adequate antibacterial activity against methanolic and hexane extracts i.e., 51.06% and 55.35% ZI at 2 mg discs⁻¹ respectively. The said fractions were found susceptible at 0.5 and 1 mg discs⁻¹ against the test microbe when matched to other fractions and control. The results also revealed that butanol and water extracts failed to show activity against *E. coli* (Figure 109).

Antibacterial strength of *Skimmia Laureola* leaves extracts against *S. aureus*

The antimicrobial activity of various solvent extracted fractions of *Skimmia Laureola* against *S. aureus* is illustrated in Figure 110. The results obtained in the study specified that ethyl acetate extract was found much more sensitive to inhibit the growth of the tested microorganism by 66.09% at the uppermost concentration of 2 mg disc⁻¹ followed by methanol extract (59.12% ZI) at the equivalent concentration. The data also proposed that the water extracted fraction from the tested leaves did not control the activity of the same bacterium at all, thus leaving 0% inhibition. Butanol extract also disclosed an outstanding amount of inhibition against *S. aureus* to variable degrees.
Figure 109: Antibacterial potential with standard deviation, of five different solvents extracted samples of leaves of *Skimmia Laureola* against *E. coli*.

Figure 110: Antibacterial potential with standard deviation, of five different solvents extracted samples of leaves of *Skimmia Laureola* against *S. aureus*.
**Antibacterial activity of *Skimmia Laureola* leaves fractions against *Xanthomonas campestris***

The antibacterial strength of various solvent extracted fractions of the shoots of *Skimmia Laureola* against *Xanthomonas campestris* is illustrated in Figure 111. *Xanthomonas campestris* disclosed complete resistance against aqueous extract presenting 0% inhibition. The topmost inhibiting value was disclosed by ethyl acetate extract (67.19% ZI) at 2 mg disc$^{-1}$ followed by crude methanolic fraction with a zone of inhibition of (62.35%) at 2 mg disc$^{-1}$. The outcomes further discovered that hexane and butanol fractions controlled significant amount of growth with 54.83% and 43% ZI at 2 mg disc$^{-1}$ concentration when related to control.

**Antifungal and anti-yeast potential of silver and gold nanoparticles (AgNPs and AuNPs) of various solvent extracted samples of *Viola pilosa* and *Skimmia Laureola***

The antifungal and anti-yeast activities of gold and silver nanoparticles of roots and shoots fractions of *Viola pilosa* and stem and leaves extracts of *Skimmia Laureola* was also a major part of the research. These activities were selected to find out the medicinal value of the nanoparticles made from plant fractions. One yeast strain and five fungal strains were used for the trial which was done through well diffusion technique.

**Antifungal and anti-yeast potential of gold nanoparticles (AuNPs) of methanolic crude roots extracts of *Viola pilosa***

The statistical results of the data about the antifungal and anti-yeast potential of gold nanoparticles of crude methanolic extracts of *Viola pilosa* roots by well diffusion technique is presented in Figure 112. The results for the crude extract AuNPs specified that the extreme level of sensitivity of 90% to the gold nanoparticles was discovered in *Paecilomyces* at 18µl disc$^{-1}$ which was followed by *Alternaria solani* with a value of 86.48% at the same concentration of 18µl disc$^{-1}$ which was then followed by the same microbe (81.08%) at a concentration of 12ul disc$^{-1}$. However lowest inhibition value was noticed at 6µl disc$^{-1}$ concentration against *C. albicans* (28.86%). The statistics also recommended that *Curvularia, Rhizopus* and *A. niger*
also presented good level of sensitivity to *Viola pilosa* crude root AuNPs while *C. albicans* revealed moderate sensitivity to the nanoparticles. The statistical data concluded that that the antifungal and antiyeast activities were purely dependent on dose. The increase in activity was noticed with an increase in the concentration level of gold nanoparticles.

**Antifungal and anti-yeast potential of gold nanoparticles (AuNPs) of aqueous root samples of *Viola pilosa***

Figure 113 illustrates the results obtained from the antifungal and anti-yeast properties of AuNPs made from the roots extract of *Viola pilosa* against five fungal strains and one yeast strain by the help of well diffusion method. The detailed results of the experiment on aqueous gold nanoparticles discovered that highest zone of inhibition (81.1%) was presented by *Paecilomyces* at 18µl disc$^{-1}$ followed by *Alternaria solani* (80.16%) at the same concentration of 18µl disc$^{-1}$. The lowest inhibitory zone of 24.43% was noticed for *C. albicans* at a concentration of 6µl disc$^{-1}$. The statistical figures also proposed that *Curvularia, Rhizopus* and *A.niger* presented a significant degree of sensitivity but *C. albicans*, showed less sensitivity to the nanoparticles. The statistical results recommended that the antifungal and antiyeast properties were dependent on dose and increase in activity was noticed upon increase in concentration of the nanoparticles.

**Antifungal and anti-yeast potential of gold nanoparticles (AuNPs) of methanolic crude shoot extracts of *Viola pilosa***

The statistical results of the data about the antifungal and anti-yeast potential of gold nanoparticles of crude methanolic extracts of *Viola pilosa* shoots by well diffusion technique is presented in Figure 114. The results for the crude extract AuNPs specified that the extreme level of sensitivity of 83.33% to the gold nanoparticles was discovered in *Paecilomyces* at 18µl disc$^{-1}$ which was followed by *Rhizopus* with value of 81.47% at the same concentration of 18µl disc$^{-1}$ which was then followed by *Paecilomyces* (80%) at a concentration of 12µl disc$^{-1}$. However lowest inhibition value was noticed at 6µl disc$^{-1}$ concentration against *C. albicans* (30%). The statistics also recommended that *Curvularia, Alternaria* and *A.niger* also presented good level of sensitivity to *Viola pilosa* crude shoot AuNPs while *C.
*albicans* revealed moderate sensitivity to the nanoparticles. The statistical data concluded that that the antifungal and antiyeast activities were purely dependent on dose. The increase in activity was noticed by increasing the concentration level of gold nanoparticles.

**Antifungal and anti-yeast potential of gold nanoparticles (AuNPs) of aqueous shoots samples of Viola pilosa**

Figure 115 illustrates the results obtained from the antifungal and anti-yeast properties of AuNPs made from the roots extract of *Viola pilosa* against five fungal strains and one yeast strain by the help of well diffusion method. The detailed results of the experiment on aqueous gold nanoparticles discovered that highest zone of inhibition (85.16%) was presented by *Rhizopus* at 18µl disc⁻¹ followed by *Paecilomyces* (84.43%) at the same concentration of 18µl disc⁻¹. The lowest inhibitory zone of 30% was noticed for *C. albicans* at a concentration of 6µl disc⁻¹. The statistical figures also proposed that *Curvularia, Alternaria* and *A. niger* presented a significant degree of sensitivity but *C. albicans*, showed less sensitivity to the nanoparticles. The statistical results recommended that the antifungal and antiyeast properties were dependent on dose and increase in activity was noticed upon increase in concentration of the nanoparticles.

**Antifungal and anti-yeast potential of gold nanoparticles (AuNPs) of methanolic crude stem samples of Skimmia laureola**

The statistical data illustrated in Figure 116 indicates the antifungal and antiyeast activity of AuNPs prepared from the crude fraction of *Skimmia laureola* stems. Generally, looking at the overall results, the stem extracted nanoparticles were highly efficient in controlling the growth against all strains under study. Comparing the control and extracts, the maximum reduction in growth of 88.27% was noticed against *Alternaria* followed by *Paecilomyces* (86.66%) at a concentration of 18µl disc⁻¹. A significant level of antifungal property was also shown by *Curvularia, Rhizopus* and *A. niger*. The lowermost value of growth inhibition 30% was observed for *C. albicans* at 6µl disc⁻¹ concentration.
Figure 111: Antibacterial potential with standard deviation, of five different solvents extracted samples of leaves of *Skimmia Laureola* against *Xanthomonas campestris*

![Antibacterial potential graph](image)

Figure 112. Antifungal and anti yeast potential, with Standard deviation of AuNPs of crude methanolic extract of *Viola pilosa* roots against five fungal and one yeast strain through well diffusion method

![Antifungal and anti yeast potential graph](image)
**Figure 113:** Antifungal and anti-yeast potential, with Standard deviation of AuNPs of aqueous extract of *Viola pilosa* roots against five fungal and one yeast strain through well diffusion method.

**Figure 114:** Antifungal and anti-yeast potential, with Standard deviation of AuNPs of crude extract of *Viola pilosa* shoots against five fungal and one yeast strain through well diffusion method.
Figure 115: Antifungal and anti-yeast potential, with Standard deviation of AuNPs of aqueous extract of Viola pilosa shoots against five fungal and one yeast strain through well diffusion method.

Figure 116: Antifungal and anti-yeast potential, with Standard deviation of AuNPs of crude methanolic extract of Skimmia laureola stem against five fungal and one yeast strain through well diffusion method.
Antifungal and anti-yeast potential of gold nanoparticles (AuNPs) of aqueous extracted stem samples of *Skimmia laureola*

Figure 117 indicates the antifungal and anti-yeast activity of nanoparticles made from the aqueous extract of *Skimmia laureola* stem. By looking at the overall results, the stem extracted nanoparticles were proficient in controlling the growth against all the strains. Increase in concentration of the nanoparticles resulted in decreasing the growth of the microscopic organism. Comparing the controls and extracts, the maximum reduction in growth of 93.33% was noticed against *C. albicans* followed by *Alterneria* (85.56%) at a concentration of 18µl disc\(^{-1}\). A significant level of antifungal property was also shown by *Curvularia, Paecilomyces* and *Rhizopus*. The lowermost value of growth inhibition 55.54% was observed for *A.niger* at 6µl disc\(^{-1}\) concentration.

**Antifungal potential of gold nanoparticles (AuNPs) of methanolic crude leaves samples of *Skimmia laureola***

The statistical data illustrated in Figure 118 indicates the antifungal and anti-yeast activity of gold nanoparticles obtained from the crude extract of *Skimmia laureola* leaves. Generally, looking at the overall results, the leaves extracted NPs (Nanoparticles) were found efficient in controlling the growth against all the strains. Comparing the control and extratcs, the maximum reduction in growth of 93.33% was noticed against *Paecilomyces* followed by *A.niger* (84.67%) at a concentration of 18µl disc\(^{-1}\). A significant level of antifungal property was also shown by *Curvularia, Rhizopus* and *Alterneria*. The lowermost value of growth inhibition 28.86% was observed for *C. albicans* at 6µl disc\(^{-1}\) concentration.

**Antifungal and anti-yeast potential of gold nanoparticles (AuNPs) of aqueous extracted leaves samples of *Skimmia laureola***

Figure 119 indicates the antifungal and anti-yeast activity of nanoparticles made from the aqueous extract of *Skimmia laureola* stem. Looking at the overall results, the stem extracted nanoparticles (NPs) were found efficient in controlling the growth against all strains. Increase in concentration of the nanoparticles resulted in decreasing the growth of the microscopic organism. Comparing the controls and extratcs, the maximum reduction of 95.53% was noticed against *Paecilomyces* at 18µl disc\(^{-1}\) followed by the same microbe with a value of 93.33% at a concentration of
12µl disc\(^{-1}\). A significant level of antifungal property was also shown by *Curvularia*, *Alternaria*, *A.niger* and *Rhizopus*. The lowermost value of growth inhibition 26.66% was observed for *C.albicans* at 6mg disc\(^{-1}\) concentration.

**Antifungal and anti-yeast potential of silver nanoparticles (AgNPs) of methanolic crude roots samples of Viola pilosa**

The statistical analysis of the data concerning the antifungal and anti-yeast potential of silver nanoparticles of crude methanolic extract of *Viola pilosa* roots by well diffusion method is is presented in Figure 120. The experimental results for the crude extract AgNPs stated that the maximum level of sensitivity of 92.78% to the nanoparticles was discovered in *A. niger* at 18µl disc\(^{-1}\) which was followed by *Rhizopus* with a value of 91.66% at the same concentration of 18µl disc\(^{-1}\) which was then followed by *Alternaria* (90.97%) at a concentration of 18µl disc\(^{-1}\). However lowest inhibition value was noticed at 6µl disc\(^{-1}\) concentration against *C. albicans* (36.66 %). The statistics also recommended that *Curvularia* and *Paecilomyces* also presented good level of sensitivity to *Viola pilosa* crude root AgNPs while *C. albicans* revealed moderate sensitivity to the nanoparticles. The statistical data concluded that that the antifungal and antiyeast activities were purely dependent on dose. The increase in activity was noticed by increasing the concentration level of AgNPs (silver nanoparticles).

**Antifungal and anti-yeast potential of silver nanoparticles (AgNPs) of aqueous roots samples of Viola pilosa**

Figure 121 illustrates the results obtained from the antifungal and antiyeast properties of AgNPs made from the roots extract of *Viola pilosa* against five fungal strains and one yeast strain by the help of well diffusion method. The detailed results of the experiment on aqueous silver nanoparticles discovered that extreme zone of inhibition (94.44%) was presented by *Rhizopus* at 18µl disc\(^{-1}\) followed by *Curvularia* (92.5%) at the same concentration of 18µl disc\(^{-1}\). The lowest inhibitory zone was noticed for *C. albicans* at a concentration of 6µl disc\(^{-1}\). The statistical figures also proposed that *Alternaria*, *A. niger* and *Paecilomyces* presented a significant degree of sensitivity but *C. albicans*, showed less sensitivity to the nanoparticles. The statistical results recommended that the antifungal and antiyeast properties were dependent on dose and increase in activity was noticed upon increase in concentration of the nanoparticles.
Antifungal and anti-yeast potential of silver nanoparticles (AgNPs) of methanolic crude shoot samples of *Viola pilosa*

The statistical analysis of the data concerning the antifungal and anti-yeast potential of silver nanoparticles of crude methanolic extract of *Viola pilosa* shoots by well diffusion method is presented in Figure 122. The experimental results for the crude extract AgNPs stated that the maximum level of sensitivity of 93.5% to the nanoparticles was discovered in *Rhizopus* at 18µl disc\(^{-1}\) which was followed by *Curvularia* with a value of 91.65% at the same concentration of 18µl disc\(^{-1}\) which was then followed by *Rhizopus* (90.72%) at a concentration of 12µl disc\(^{-1}\). However lowest inhibition value was noticed at 6µl disc\(^{-1}\) concentration against *C. albicans* (28.86%). The statistics also recommended that *Paecilomyces*, *Alternaria* and *A. niger* also presented good level of sensitivity to *Viola pilosa* shoot crude AgNPs while *C. albicans* revealed moderate sensitivity to the nanoparticles. The statistical data concluded that that the antifungal and antiyeast activities were purely dependent on dose. The increase in activity was noticed by increasing the concentration level of AgNPs (silver nanoparticles).

Antifungal and anti-yeast potential of silver nanoparticles (AgNPs) of aqueous shoot samples of *Viola pilosa*

Figure 123 illustrates the results obtained from the antifungal and anti-yeast properties of AgNPs made from the shoots extract of *Viola pilosa* against five fungal strains and one yeast strain by the help of well diffusion method. The detailed results of the experiment on aqueous silver nanoparticles discovered that extreme zone of inhibition (95%) was presented by *Curvularia* at 18µl disc\(^{-1}\) followed by *A. niger* (94.59%) at the same concentration of 18µl disc\(^{-1}\). The lowest inhibitory zone of 26.66% was noticed for *C. albicans* at a concentration of 6µl disc\(^{-1}\). The statistical figures also proposed that, *Rhizopus* and *Paecilomyces* presented a significant degree of sensitivity but *C. albicans*, showed less sensitivity to the nanoparticles. The statistical results recommended that the antifungal and antiyeast properties were dependent on dose and increase in activity was noticed upon increase in concentration of the nanoparticles.
Figure 117: Antifungal and anti yeast potential, with Standard deviation of AuNPs of aqueous extract of *Skimmia laureola* stem against five fungal and one yeast strain through well diffusion method.

Figure 118: Antifungal and anti yeast potential, with Standard deviation of AuNPs of crude extract of *Skimmia laureola* leaves against five fungal and one yeast strain through well diffusion method.
Figure 119: Antifungal and anti yeast potential, with Standard deviation of AuNPs of aqueous extract of *Skimmia laureola* leaves against five fungal and one yeast strain through well diffusion method.

Figure 120: Antifungal and antiyeast potential, with Standard deviation of AgNPs of crude methanolic extract of *Viola pilosa* roots against five fungal and one yeast strain through well diffusion method.
**Figure 121:** Antifungal and anti-yeast potential, with Standard deviation of AgNPs of aqueous extract of *Viola pilosa* roots against five fungal and one yeast strain through well diffusion method.

**Figure 122:** Antifungal and anti-yeast potential, with Standard deviation of AgNPs of crude methanolic extract of *Viola pilosa* shoots against five fungal and one yeast strain through well diffusion method.
**Figure 123:** Antifungal and anti-yeast potential, with Standard deviation of AgNPs of aqueous extract of *Viola pilosa* shoots against five fungal and one yeast strain through well diffusion method

**Figure 124:** Antifungal and anti-yeast potential, with Standard deviation of AgNPs of crude methanolic extract of *Skimmia laureola* stem against five fungal and one yeast strain through well diffusion method
Antifungal and anti-yeast potential of silver nanoparticles (AgNPs) of methanolic crude stem samples of *Skimmia laureola*

The statistical data illustrated in Figure 124 indicates the antifungal and anti-yeast activity of nanoparticles obtained from the crude extract of *Skimmia laureola* stems. Generally, looking at the overall results, the stem extracted NPs (Nanoparticles) were found efficient in controlling the growth against all the strains. Comparing the control and extratcs, the maximum reduction in growth of 95.53% was noticed against *Paecilomyces* followed by *Alternaria* (93.67%) at a concentration of 18µl disc¹. A significant level of antifungal property was also shown by *Curvularia*, *Rhizopus* and *A. niger*. The lowermost value of growth inhibition 40% was observed for *C. albicans* at 6µl disc¹ concentration.

Antifungal and anti-yeast potential of silver nanoparticles (AgNPs) of aqueous extracted stem samples of *Skimmia laureola*

Figure 125 indicates the antifungal and anti-yeast activity of nanoparticles made from the aqueous extract of *Skimmia laureola* stems. Looking at the overall results, the stem extracted nanoparticles (NPs) were found efficient in controlling the growth against all the strains. Increase in concentration of the nanoparticles resulted in decreasing the growth of the microscopic organism. Comparing the controls and extratcs, the maximum reduction in growth of 94.49% was noticed against *A. niger* and *Alterneria* followed by *Rhizopus* (92.58%) at a concentration of 18µl disc¹. A significant level of antifungal property was also shown by *Curvularia* and *Paecilomyces*. The lowermost value of growth inhibition 27.76% was observed for *C. albicans* at 6µl disc¹ concentration.

Antifungal and anti-yeast potential of silver nanoparticles (AgNPs) of methanolic crude leaves samples of *Skimmia laureola*

The statistical data illustrated in Figure 126 indicates the antifungal and anti-yeast activity of nanoparticles obtained from the crude extract of *Skimmia laureola* leaves. Generally, looking at the overall results, the stem extracted NPs (Nanoparticles) were found proficient in controlling the growth against all strains. Comparing the control and extratcs, the maximum reduction in growth of 93.67% was
noticed against *A.niger* and *A.alterneria* followed by *Curvularia* (89.8%) at a concentration of 18µl disc\(^{-1}\). A significant level of antifungal property was also shown by *Rhizopus, Alterneria* and *Paecilomyces*. The lowermost value of growth inhibition 28.86% was observed for *C. albicans* at 6µl disc\(^{-1}\) concentration.

**Antifungal and anti-yeast potential of silver nanoparticles (AgNPs) of aqueous extracted leaves samples of *Skimmia laureola***

Figure 127 indicates the antifungal and anti-yeast activity of nanoparticles made from the aqueous extract of *Skimmia laureola* leaves. Looking at the overall results, the leaves extracted NPs (Nanoparticles) were truly capable in controlling the growth against all the strains. Increase in concentration of the nanoparticles resulted in decreasing the growth of the microscopic organism. Comparing the controls and extracts, the maximum reduction in growth of 91.89% was noticed against *A.niger* followed by *Paecilomyces* (90%) at a concentration of 18µl disc\(^{-1}\). A significant level of antifungal property was also shown by *Curvularia, Alterneria* and *Rhizopus*. The lowermost value of growth inhibition 26.66% was observed for *C. albicans* at 6µl disc\(^{-1}\) concentration.

**Antifungal potential of plants extracts**

**Antifungal potential of solvent fractions obtained from roots of *Viola pilosa* against *Candida albicans***

The results of Figure 128 show the anti yeast activity of *Viola pilosa* root extracts against *C. albicans*. The data obtained from disc diffusion assay exposed that different solvent extracted samples measured varying degree of growth inhibition at all the tested concentrations. The results revealed that growth reduction of the tested microbe was dose dependent. Maximum growth inhibition of 61.83% was noted by hexane fraction at 2 mg disc\(^{-1}\) concentration followed by butanol (58.5%) at the equivalent concentration. Similarly, least possible activity (23.3% ZI) against the same microbe was detected for aqueous fraction at 0.5 mg disc\(^{-1}\) when matched with other samples and controls.
Figure 125: Antifungal and anti yeast potential, with Standard deviation of AgNPs of aqueous extract of *Skimmia laureola* stem against five fungal and one yeast strain through well diffusion method.

Figure 126: Antifungal and anti yeast potential, with Standard deviation of AgNPs of crude methanolic extract of *Skimmia laureola* leaves against five fungal and one yeast strain through well diffusion method.
**Figure 127:** Antifungal and anti yeast potential, with Standard deviation of AgNPs of aqueous extract of *Skimmia laureola* leaves against five fungal and one yeast strain through well diffusion method.

**Figure 128:** Anti yeast potential of *Viola pilosa* root extracts against *C. albicans.*
Antifungal strength of solvent extracted fractions from roots of *Viola pilosa* against *Alternaria solani*

Data shown in Figure 129 indicated that various solvent extracted fractions reduced the growth of *A. solani* and this growth reduction was dose dependent. The highest growth inhibition (95.91%) was recorded for methanolic fraction at 2 mg disc\(^{-1}\) followed by 94.13% ZI noted by the same fraction at 1 mg disc\(^{-1}\). The outcomes also recommended that the other tested fractions were also capable in controlling the growth of the same microorganism measuring different zones of inhibition at all concentration. It is also clear from the result that lowest antifungal activity of 66.18% was revealed by hexane extracted fraction at 0.5 mg disc\(^{-1}\).

Antifungal potential of solvent extracted fractions from roots of *Viola pilosa* against *Curvularia*

The outcomes revealed that the growth of *Curvularia* was inhibited by various solvent fractions at all three tested concentrations (Figure 130). Maximum extent of inhibition was presented by aqueous extracted fraction (88.13% ZI) at 2 mg disc\(^{-1}\) followed by crude fraction (74.10% ZI) at 2 mg disc\(^{-1}\). The consequences also directed that butanol fraction was not much competent in controlling the growth of the microscopic organism under study, at 0.5 mg disc\(^{-1}\) measuring 32.44% ZI.

Antifungal potential of solvent extracted fractions from roots of *Viola pilosa* against *A. niger*

The results indicated that *A. niger* was also susceptible to different solvent fractions of the tested plant at all concentrations. Maximum growth reduction of 48.18% was recorded for ethyl acetate at the uppermost concentration of 2 mg disc\(^{-1}\) followed by 47.27% of the butanol extract at 2mg disc\(^{-1}\). Minimum activity of 30.16% was noted for the same microbe by crude extracted samples at the lowest concentration of 0.5 mg disc\(^{-1}\) (Figure 131).
Figure 129: Antifungal potential of *Viola pilosa* root extracts against *A. solani*

Figure 130: Antifungal potential of *Viola pilosa* root extracts against *Curvularia*
**Figure 131:** Antifungal potential of *Viola pilosa* root extracts against *A.niger*

**Figure 132:** Antifungal potential of *Viola pilosa* root extracts against *Paecelomyces*
Antifungal strength of solvent fractions from roots of *Viola pilosa* against *Paecilomyces*

The strength of different fractions extracted from roots of *Viola pilosa* against *Paecilomyces* is displayed in Figure 132. Examination of the records discovered that hexane extracted fraction was highly capable in controlling the development of the test fungus by 95.60% at the highest concentration of 2 mg disc\(^{-1}\) followed by butanol extracted sample (93.84% ZI) at the same concentration. Hexane, crude, aqueous and ethyl acetate extracts also reduced the growing power of *Paecilomyces* to varying degree.

Antifungal potential of solvent extracted fractions from roots of *Viola pilosa* against *Rhizopus*

The results of Figure 133 show the antifungal activity of *Viola pilosa* root extracts against *Rhizopus*. The data obtained from well diffusion assay exposed that different solvent extracted samples measured varying degree of growth inhibition at all the tested concentrations. The results revealed that growth reduction of the tested microbe was dose dependent. Maximum growth inhibition of 92.33% was noted by hexane fraction at 2 mg disc\(^{-1}\) concentration followed by n-butanol (82.65%) at the equivalent concentration. Similarly, lowermost activity (46.5% ZI) against the same microbe was measured by aqueous extracted samples at 0.5 mg disc\(^{-1}\) when matched to control and other samples.

Antifungal potential of solvent fractions obtained from shoots of *Viola pilosa* against *C. albicans*

The antifungal strength of solvent fractions obtained from the shoots of *Viola pilosa* against *C. albicans* is shown in Figure 134. The tested microbe presented high zone of inhibition to butanol, ethyl acetate, hexane and methanol extracted fractions. The maximum inhibitory zone of 41.1% was measured by ethyl acetate and hexane fractions at a concentration of 2 mg disc\(^{-1}\) and the lowermost by aqueous fraction 27.2% at a concentration of 0.5 mg disc\(^{-1}\).
Figure 133: Antifungal potential of *Viola pilosa* root extracts against *Rhizopus*

Figure 134: Antifungal potential with standard deviation, of five different solvents extracted samples of shoot of *Viola pilosa* against *C.albicans*
Antifungal strength of solvent fractions obtained from shoots of *Viola pilosa* against *Alternaria solani*

Figure 135 explains the probable antimicrobial strength of *Viola pilosa* shoots extracts against *Alternaria*. The outcomes showed that the different fractions unveiled variable degree of antifungal strength on the test microorganism. The data revealed that the reduction in growth of the test microscopic organism was dose dependent. Amongst all fractions, the uppermost zone of inhibition of 96.37 was measured by aqueous extract at 2 mg disc\(^{-1}\) concentration followed by crude (95.02\%) at the similar concentration. However, minimum activity of 79.70 \% was shown by hexane extracted samples against the same microbe at 0.5 mg disc\(^{-1}\) concentration when matched to controls.

Antifungal potential of solvent fractions obtained from shoots of *Viola pilosa* against *Curvularia*

The data illustrated that the Crude methanolic fraction and other solvent extracted samples inhibited the growth of *Curvularia* (Figure 136). The highest zone of inhibition of 88.57\% against *Curvularia* was recorded for hexane at the maximum concentration of 2 mg discs\(^{-1}\) followed by crude with a zone of inhibition of 84.63\% at the same concentration. Aqueous, n-butanol and ethyl acetate displayed adequate inhibition results. The lowest inhibiting value of 44.28\% was noted for ethyl acetate at a concentration of 0.5 mg discs\(^{-1}\).

Antifungal potential of solvent fractions obtained from shoots of *Viola pilosa* against *A.niger*

The antifungal activity of various solvent extracted fractions of *Viola pilosa* against *A. niger* is illustrated in Figure 137. The results obtained in the study designated that n-butanol fraction was more sensitive in inhibiting the growth of the tested microscopic organism by 51.78\% at the maximum concentration of 2 mg disc\(^{-1}\) followed by ethyl acetate extract (49.97\% ZI) at the similar concentration. Methanolic, hexane and aqueous fractions also revealed good inhibiting activity against *A.niger* to varying degrees.
Figure 135: Antifungal potential with standard deviation, of five different solvents extracted samples of shoot of *Viola pilosa* against *Alterneria solani*.

Figure 136: Antifungal potential with standard deviation, of five different solvents extracted samples of shoot of *Viola pilosa* against *Curvularia*.
**Figure 137:** Antifungal potential with standard deviation, of five different solvents extracted samples of shoot of *Viola pilosa* against *A.niger*

**Figure 138:** Antifungal potential with standard deviation, of five different solvents extracted samples of shoot of *Viola pilosa* against *Paecilomyces lilacinus*
Antifungal strength of solvent fractions from shoots of *Viola pilosa* against *Paecilomyces lilacinus*

*Paecilomyces lilacinus* showed maximum susceptibility to n-hexane extracted fractions measuring highest growth inhibition activity of 95.89% at the higher concentration of 2 mg disc$^{-1}$ followed by 93.84% and 90.76% inhibiting value at 1 and 0.5 mg discs$^{-1}$ respectively. The outcomes directed that *P. lilacinus* also exhibited moderate antifungal activity against butanol and crude methanolic and aqueous extracts i.e., 94.71%, 92.97% and 91.65% ZI at 2 mg discs$^{-1}$ respectively. These fractions were also susceptible at 1 and 0.5 mg discs$^{-1}$ against the test microbe when matched to remaining samples and control (Figure 138).

Antifungal potential of solvent fractions from from shoots of *Viola pilosa* against *Rhizopus*

The antifungal strength of different fractions extracted from shoots of *Viola pilosa* against *Rhizopus* is illustrated in Figure 139. Maximum inhibition was showed by aqueous extracts (87.08% ZI) at 2 mg disc$^{-1}$ followed by butanol extracts with a zone of inhibition of (85.83%) at 2 mg disc$^{-1}$. The experimental results also discovered that crude, ethyl acetate and hexane fractions possessed significant activity of 83.29%, 82.62% and 78.45 % ZI at 2 mg disc$^{-1}$ concentration against *Rhizopus* when matched to control.

Antifungal potential of solvent fractions from leaves of *Skimmia Laureola* against *Candida Albicans*.

The results of Figure 140 indicate the antifungal activity of *Skimmia laureola* leaves extracts against *C.albicans*. The statistical data exposed that solvent extracted samples presented different degree of growth inhibition at all the concentrations under study. The results revealed that growth reduction of the tested microscopic organism was dose dependent. Uppermost inhibiting activity of 63.86% was noted for hexane samples at 2 mg disc$^{-1}$ concentration followed by ethyl acetate (61.1%) at the same concentration. Similarly, minimum activity (33% ZI) against the same microbe was recorded for butanol fraction at 0.5 mg disc$^{-1}$ when matched to remaining samples and
control. The results also revealed that aqueous extract was unable to show activities against *C.albicans*.

**Antifungal strength of different fracions from leaves of Skimmia Laureola against Alterneria**

The strength of solvent fractions obtained from leaves of *Skimmia Laureola* against *Alterneria* is presented in Figure 141. Consideration of the results discovered that aqueous and butanol fractions were efficent in controlling the growth and development of the test fungus by 99.54% at the highest concentration of 2 mg disc\(^{-1}\) followed by crude methanolic extracted sample (96.83% ZI) at the equivalent concentration. The results further discovered that ethyl acetate and hexane extracts also reduced the growth of *Alterneria* to varying degrees.

**Antifungal potential of solvent fractions from leaves of Skimmia Laureola against Curvularia**

Data presented in Figure 142 specified that solvent fractions from leaves reduced the growth of *Curvularia* and this growth reduction was dose dependent. The highest growth inhibition (91.22%) was recorded for butanol at 2 mg disc\(^{-1}\) followed by 89.57% ZI noted for aqueous fraction at 2 mg disc\(^{-1}\). The outcomes also recommended that the other tested fractions also controlled the growth of the same microscopic organism measuring different zones of inhibition at all concentration. It is also obvious from the result that lowest antifungal strength of 54.57% was revealed by crude extracted fraction at 0.5 mg disc\(^{-1}\).

**Antifungal potential of solvent fractions from leaves of Skimmia Laureola against A.niger**

The antifungal strength of solvent fractions from the roots of *Skimmia Laureola* against *A.niger* specified that the test microscopic organism showed complete susceptibility to all the solvent fractions and was capable to show good amount of activities at the tested concentrations (Figure 143). Maximum inhibition value of 98.18% was noticed for aqueous extract at a concentration of 2 mg disc\(^{-1}\) followed by ethyl acetate and butanol with 88.72% and 87.81% at the equivalent concentration of 2 mg disc\(^{-1}\).
Figure 139: Antifungal potential with standard deviation, of five different solvents extracted samples of shoot of *Viola pilosa* against *Rhizopus*

Figure 140: Antifungal potential with standard deviation, of five different solvents extracted samples of stem of *Skimmia Laureola* against *C.albicans*
Figure 141: Antifungal potential with standard deviation, of five different solvents extracted samples of stem of *Skimmia Laureola* against *Alterneria*

![Graph](image1)

Figure 142: Antifungal potential with standard deviation, of five different solvents extracted samples of stem of *Skimmia Laureola* against *Curvularia*

![Graph](image2)
Figure 143: Antifungal potential with standard deviation, of five different solvents extracted samples of stem of *Skimmia Laureola* against *A. niger*.

Figure 144: Antifungal potential with standard deviation, of five different solvents extracted samples of stem of *Skimmia Laureola* against *Paecilomyces*.
Antifungal potential of solvent fractions obtained from leaves of *Skimmia Laureola* against *Paecilomyces*

The outcomes illustrated that the Crude methanolic and other solvent extracted samples inhibited the growth of *Paecilomyces* (Figure 144). The highest zone of inhibition of 96.1% against *Paecilomyces* was recorded for hexane at the maximum concentration of 2 mg discs$^{-1}$ followed by 94.96% inhibition value of the ethyl acetate fraction at a concentration of 2 mg discs$^{-1}$. The results showed that butanol, aqueous and methanolic extracts also displayed reasonable activities against the fungus under study.

Antifungal potential of solvent fractions from stem of *Skimmia Laureola* against *Rhizopus*

The antifungal strength of solvent extracted fractions from the leaves of *Skimmia Laureola* against *Rhizopus* is shown in Figure 145. The tested microbe presented high zone of inhibition to butanol, aqueous, ethyl acetate and methanolic fractions. The maximum inhibitory zone of 91.19% was recorded for aqueous fraction at a concentration of 2 mg disc$^{-1}$ and the lowermost by crude samples 68.02% at a concentration of 0.5 mg disc$^{-1}$.

Antifungal potential of different solvent fractions samples from stem of *Skimmia Laureola* against *Candida Albicans*

Figure 146 illuminates the probable antifungal strength of *Skimmia Laureola* stem extract against *C. albicans*. The outcomes indicated that different fractions presented variable degree of antifungal activities on the microorganism tested. The data revealed that the reduction in growth of the test microscopic organism was totally dependent on dose. Amongst all fractions, the uppermost inhibition of 63.33% was measured by hexane fraction at 2 mg disc$^{-1}$ concentration followed by ethyl acetate (66.1%) at the similar concentration. However, low activity of 30.53% was presented by butanol samples at 0.5 mg disc$^{-1}$ concentration against the same microbe when compared with controls.
Figure 145: Antifungal potential with standard deviation, of five different solvents extracted samples of stem of *Skimmia Laureola* against *Rhizopus*

Figure 146: Antifungal potential with standard deviation, of five different solvents extracted samples of stem of *Skimmia Laureola* against *C. albicans*
Antifungal strength of solvent fractions from stem of *Skimmia Laureola* against *Rhizopus*

*Rhizopus* showed maximum susceptibility to butanol extracted fractions measuring highest growth inhibition of 97.72% at the higher concentration of 2 mg disc$^{-1}$ followed by 92.32.% and 86.91% zone of inhibition at 1 and 0.5 mg discs$^{-1}$ respectively when compared with other extracts and controls. The data indicated that *Rhizopus* also exhibited adequate antifungal activity against aqueous, crude, hexane and ethyl acetate extracts i.e., 95.91%, 84.21%, 77.45% and 74.29% ZI at 2 mg discs$^{-1}$ respectively. These fractions also remained susceptible at 1 and 0.5 mg discs$^{-1}$ against the test mircobe when matched to remaining samples and control (Figure 147).

Antifungal potential of solvent fractions from stem of *Skimmia Laureola* against *Curvularia*

The antifungal potential of various solvent fractions of *Skimmia Laureola* against *Curvularia* is illustrated in Figure 148. The results obtained in the study indicated that aqueous extracted sample was pretty much sensitive to inhibit the growth of the tested microscopic organism by 91.22% at the uppermost concentration of 2 mg disc$^{-1}$ followed by methanol extracted sample (88.72% ZI) at the equivalent concentration. The data also proposed that the ethyl acetate extracted sample from the tested leaves showed lowest activity of 70.57% for the same fungus at lowest concentration of 0.5mg disc$^{-1}$. Butanol extracted fraction also revealed good inhibiting activity against *Curvularia*.

Antifungal potential of various solvent fractions from stem of *Skimmia Laureola* against *Alterneria*

The antifungal potential of solvent fractions from the stem of *Skimmia Laureola* against *Alterneria* is illustrated in Figure 149. Maximum growth reduction was noticed for butanol and aqueous fractions (98.62% ZI) at 2 mg disc$^{-1}$ followed by crude methanolic fraction with a zone of inhibition of (97.29%) at 2 mg disc$^{-1}$. The outcomes also discovered that hexane and ethyl acetate fractions exposed significant strength of 85.10% and 77 % ZI at 2 mg disc$^{-1}$ concentration against *Alterneria* when associated to control.
Figure 147: Antifungal potential with standard deviation, of five different solvents extracted samples of stem of *Skimmia Laureola* against *Rhizopus*

Figure 148: Antifungal potential with standard deviation, of five different solvents extracted samples of stem of *Skimmia Laureola* against *Curvularia*
Figure 149: Antifungal potential with standard deviation, of five different solvents extracted samples of stem of *Skimmia Laureola* against *Alterneria*

Figure 150: Antifungal potential with standard deviation, of five different solvents extracted samples of stem of *Skimmia Laureola* against *Paecilomyces*
Antifungal potential of solvent fractions from stem of *Skimmia Laureola* against *Paecilomyces*

The results indicated that *Paecilomyces* was also susceptible to different solvent fractions of tested plant at all concentration. Maximum inhibiting strength of 99.41% was recorded for ethyl acetate at the uppermost concentration of 2 mg disc⁻¹ followed by 98.24% each of the hexane extract at the equivalent concentration of 2 mg disc⁻¹. The lowest activity were noted for the same microbe by aqueous and crude extracted samples giving the values of 83.31% and 78.72% at a given concentration of 0.5 mg disc⁻¹ (Figure 150).

**Antifungal potential of solvent fractions from stem of *Skimmia Laureola* against *Niger***

Data shown in Figure 151 indicated that solvent fractions from the stems controlled the growth of *A. niger* and this growth reduction was dose dependent. The highest growth inhibition (95.91%) was measured by aqueous samples at 2 mg disc⁻¹ followed by 90.51% ZI noted by the crude fractions at 2 mg disc⁻¹. The data consequences also recommended that the other tested fractions also controlled the growth of the same microscopic organism measuring different zones of inhibition at all concentration. It is also clear from the result that lowest antifungal activity of 69.35% was presented by ethyl acetate extracted fraction at 0.5 mg disc⁻¹.

**DPPH Radical Scavenging Activity of Nanoparticles and Plant fractions**

The antioxidant strength of different extracts and their respective nanoparticles from the roots and shoots of *Viola* as well as from the leaves and stem of *Skimmia* was evaluated in DPPH radical scavenging bioassay. Each extract was used in four different concentrations (25, 50, 125 and 250 µg/ml) and their radical scavenging activity was assessed against the 98.95% activity exhibited by the standard, Gallic acid (500 µg/ml).
Figure 151: Antifungal potential with standard deviation, of five different solvents extracted samples of stem of *Skimmia Laureola* against *A.niger*.

Figure 152: DPPH Radical Scavenging activity of gold nanoparticles extracted from *Viola* root crude, *Viola* root aqueous, *Viola* shoot crude and *Viola* shoot aqueous fractions.
Radical Scavenging Activity of aqueous and crude extracted gold nanoparticles from Viola roots and shoots

All tested concentrations of all gold nanoparticle types were active in scavenging free radicals when evaluated in DPPH radical scavenging assay (Figure 152). Viola roots aqueous extracted nanoparticles with activities measuring to 58.58%, 54.78%, 53.24% and 52.51% at 25, 50, 125 and 250 µg/ml respectively were by far the most potent of the tested extracts in scavenging free radicals. Viola root crude (57.2% at 250 µg/ml), Viola shoot aqueous (55.43% at 250 µg/ml) and Viola shoot crude (54.58% at 250 ug/ml) followed suit in a sequential manner. Viola shoot crude fraction, on the other hand, measured the least activities in comparison to the other tested nanoparticles (54.58%, 53.19%, 52.86% and 50.01% activity at 25, 50, 125 and 250 µg/ml respectively).

Radical Scavenging Activity of aqueous and crude extracted silver nanoparticles from Viola roots and shoots

The data, graphically represented in Figure 153, revealed the presence of antioxidant activity for all tested silver nanoparticles at each concentration used. The least potent among the tested nanoparticles turned out to be Viola root aqueous extract (72.87%, 68.8%, 52.68% and 45.34% at 250, 125, 50 and 25 µg/ml respectively) while the best antioxidant activity was revealed by the nanoparticles extracted with Viola shoots aqueous fraction measuring activities of 76.67%, 71.82%, 66.44% and 50% at 250, 125, 50 and 25 µg/ml respectively. The remaining extracts in the sequence of descending activities were Viola shoot crude fraction (74.63% at 250 µg/ml), Viola root crude extract (74.5% at 250 µg/ml) and Viola root aqueous fraction (72.87% at 250 µg/ml).

Radical Scavenging Activity of aqueous and crude extracted silver nanoparticles from the leaves and stem Skimmia laureola

All the silver nanoparticles extracted from the leaves and stem of Skimmia were active in scavenging free radicals at each of the four concentrations chosen for experiment (Figure 154). The best radical scavenging potential was depicted by Skimmia stem aqueous fraction with activities measuring to 80.86%, 76.99%, 74.37%
and 68.47% at 250, 125, 50 and 25 µg/ml respectively. 

**Radical Scavenging Activity of aqueous and crude extracted gold nanoparticles from the leaves and stem Skimmia laureola**

Four samples in different gold nanoparticles were also extracted from the stem and leaves of Skimmia and all showed antioxidant activity at each tested concentration (Figure 155). Skimmia stem aqueous fraction revealed 75.49%, 66.44%, 63.3% and 56.88% activity at 250, 125, 50 and 25 µg/ml respectively and was at the top of the list in scavenging free radicals. The next in line according to the order of descending activity were Skimmia leaves aqueous, Skimmia leaves crude, butanol, aqueous and Skimmia stem crude fractions which measured an activity of 69.79%, 67.69% and 62.12% respectively at 250 µg/ml. Skimmia stem crude extract measured 61.12%, 59.37%, 55.24% and 52.7% activity at 250, 125, 50 and 25 µg/ml respectively and, hence, turned out to be the least potent among the tested nanoparticles extracts in scavenging free radicals.

**Radical Scavenging Activity of Extracts from Viola Roots**

All tested concentrations of each extract were active in scavenging free radicals when evaluated in DPPH radical scavenging assay (Figure 156). Ethyl acetate fraction with activities measuring to 73.26%, 94.62%, 95.28% and 99.21% at 25, 50, 125 and 250 µg/ml respectively was by far the most potent of the tested extracts in scavenging free radicals. Butanol (95.87% at 250 µg/ml), aqueous (93.51% at 250 µg/ml) and methanol (68.41% at 250 µg/ml) followed suit in a sequential manner. Hexane fraction, on the other hand, measured the least activities in comparison to the other tested extracts (41.34%, 42.33%, 54.32% and 68.41% activity at 25, 50, 125 and 250 µg/ml respectively).
Figure 153: DPPH Radical Scavenging activity of silver nanoparticles extracted from *Viola* root crude, *Viola* root aqueous, *Viola* shoot crude and *Viola* shoot aqueous fractions.

Figure 154: DPPH Radical Scavenging activity of silver nanoparticles extracted from *Skimmia* leaves crude, *Skimmia* leaves aqueous, *Skimmia* stem crude and *Skimmia* stem aqueous fractions.
Figure 155: DPPH Radical Scavenging activity of gold nanoparticles extracted from *Skimmia* leaves crude, *Skimmia* leaves aqueous, *Skimmia* stem crude and *Skimmia* stem aqueous fractions.

Figure 156: DPPH Radical Scavenging activity of crude methanol, hexane, ethyl acetate, butanol and aqueous extracts from the roots of *Viola*. 
Radical Scavenging Activity of Extracts from *Viola* Shoots

The data, graphically represented in Figure 157, revealed the presence of antioxidant activity for all tested extracts at each concentration used. The least potent amongst the test extracts was found to be hexane extract (66.12%, 51.37%, 40.95% and 39.90% at 250, 125, 50 and 25 µg/ml respectively) while the best antioxidant activity was revealed by the sample extracted with butanol measuring activities of 95.87%, 95.80%, 81.32% and 60.02% at 250, 125, 50 and 25 µg/ml respectively. The remaining extracts in the sequence of descending activities were aqueous fraction (93.51% at 250 µg/ml), methanol extract (89.71% at 250 µg/ml) and ethyl acetate fraction (86.23% at 250 µg/ml).

Radical Scavenging strength of Fractions from *Skimmia* Leaves

All fractions taken out from the leaves of *Skimmia* were found proficient in scavenging free radicals at each of the four concentrations chosen for the experiment (Figure 158). The best radical scavenging potential was depicted by butanol fraction with activities measuring to 84.01%, 59.37%, 52.49% and 44.69% at 250, 125, 50 and 25 µg/ml respectively. Aqueous fraction followed suit with an activity of 62.64% at 250 µg/ml which in turn was followed by ethyl acetate and crude extracts with 60.55% and 54.98% activity respectively at the highest concentration used. The lowest activities were noticed at 52.88%, 48.88%, 42.92% and 41.21% at 250, 125, 50 and 25 µg/ml respectively which were exhibited by the sample extracted with hexane.

Radical Scavenging Activity of Extracts from *Skimmia* Stem

Five samples in different solvents were also extracted from the stem of *Skimmia* and all showed antioxidant activity at each tested concentration (Figure 159). Ethyl acetate fraction revealed 84.40%, 69.26%, 56.68% and 48.72% activity at 250, 125, 50 and 25 µg/ml respectively and was at the top of the list in scavenging free radicals. The next in line according to the order of descending activity were butanol, aqueous and hexane fractions which measured an activity of 75.22%, 66.44% and 62.9% respectively at 250 µg/ml. Crude methanol extract measured 61.99%, 48.95%, 42.95% and 39.44% activity at 250, 125, 50 and 25 µg/ml respectively and, hence, turned out to be the least potent among the tested extracts in scavenging free radicals.
Figure 157: DPPH Radical Scavenging activity of crude methanol, hexane, ethyl acetate, butanol and aqueous extracts from the shoots of Viola.

Figure 158: DPPH Radical Scavenging activity of crude methanol, hexane, ethyl acetate, butanol and aqueous extracts from the leaves of Skimmia.
**Figure 159:** DPPH Radical Scavenging activity of crude methanol, hexane, ethyl acetate, butanol and aqueous extracts from the stem of *Skimmia*.

**Table 3:** Phytochemical analysis of methanolic extracts from roots of *Viola pilosa*.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Alkaloids</th>
<th>Proteins</th>
<th>Tannins</th>
<th>Carbohydrates</th>
<th>Sterols</th>
<th>Flavonoids</th>
<th>Saponins</th>
<th>Fats and Oils</th>
</tr>
</thead>
<tbody>
<tr>
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<td>+++</td>
<td>+++</td>
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<td>+++</td>
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<tr>
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<td>+</td>
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<td>++</td>
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<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

+++ : shows the presence in abundance  
++ : shows presence in moderate quantity  
+ : shows presence but in less amount  
_ : shows complete absence of the compound
OBJECTIVE 4:

Phyto chemistry of solvent fractions from roots of Viola pilosa

In the present-day research different solvent fractions from the roots of Viola pilosa were also screened for alkaloids, tannins, fats and oils, proteins, carbohydrates, sterols, flavonoids and saponins (Table 3). Primary analysis of phytochemistry of the crude fraction exposed traces of proteins, moderate quantity of alkaloids and saponins and high quantity of tannins, fats and oils, carbohydrates, sterols and flavonoids. The study presented that hexane was found negative for tannins, alkaloids and proteins and was rich in fats, oils and flavonoids. The butanol extracted fraction presented good content of tannins, carbohydrates and saponins and moderate content of fats, oils, sterols, flavonoids and alkaloids. The results further suggested that ethyl acetate was found negative for fats, alkaloids and oils along with some traces of proteins, saponins and carbohydrates. In the present study it was demonstrated that water extracted fraction exhibited high content of flavonoids, fats and oils and a good content of tannins, carbohydrates and sterols. The lowest level was found for proteins, alkaloids and saponins.

Phyto chemistry of solvent fractions from shoots of Viola pilosa

In the current study different solvent extracted samples from the shoots of Viola pilosa were also exposed to phytochemical investigation for the presence of Alkaloids, flavonoids, proteins, fats, oils, tannins, carbohydrates, sterols and saponins conferring to the systematic analysis practices (Table 4). Methanolic fraction turned out to be vigorous in sterols, carbohydrates and tannins. Phytochemistry of the crude fraction also presented reasonable amount of fats, alkaloids flavonoids, oils and a good extent of saponins and proteins. The outcomes also pointed out that hexane fraction disclosed negative effects for oils, alkaloids, fats, tannins and saponins. In the same way, ethyl acetate also turned out to be negative for saponins and alkaloids. The fraction obtained from butanol presented remarkable consequences by unveiling a very good level of phytochemistry. The data also specified the richness of aqueous fraction in tannins, alkaloids, carbohydrates, oils, fats and sterols followed by adequate amount of flavonoids and proteins.
### Table 4: Phytochemical profile of solvent extracted samples from shoots of *Viola pilosa.*

<table>
<thead>
<tr>
<th>Extract</th>
<th>Alkaloids</th>
<th>Proteins</th>
<th>Tannins</th>
<th>Carbohydrates</th>
<th>Sterols</th>
<th>Flavonoids</th>
<th>Saponins</th>
<th>Fats and Oils</th>
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<td>+++</td>
<td>++</td>
<td>+</td>
<td>_</td>
<td>+++</td>
</tr>
</tbody>
</table>

+++ shows the presence in abundance  
++ : shows presence in moderate quantity  
+ : shows presence but in less amount  
_ : shows complete absence of the compound

### Table 5: Different phytochemicals present in stem of *Skimmia laureola*

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Crude</th>
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<th>Hexane</th>
<th>E.acetate</th>
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</tr>
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<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

+++ : presence in abundance  
++ : presence in moderate quantity  
+ : presence but in less amount  
_ : complete absence of the compound
Phyto chemical study of solvent fractions from stem samples of *Skimmia laureola*

The primary results achieved from phytochemical study of the *Skimmia laureola* stem is shown in Table 5. The qualitative tests on the stems discovered the existence of secondary metabolites in crude and other extracts under study, such as alkaloids, flavonoids, proteins, fats, oils, tannins, carbohydrates, sterols and saponins and glycosides. The samples were abundant in tannins, carbohydrates, sterols, proteins and lipids. The results obtained from the stem of *S. laureola* unveiled the existence of sterols, flavonoids and tannins in all the fractions under study whereas saponins were observed in all the extracts excluding ethyl acetate extracted samples. However, lipids were found abundant in crude and hexane extracted fractions and were found absent in n-butanol, aqueous and ethyl acetate fractions. The phytochemistry of the stem extracts also demonstrated the moderate occurrence of alkaloids in crude and butanol extracts of *Skimmia laureola* while showing negative results for ethyl acetate, n-hexane and aqueous fractions respectively. Thus, the overall presence of phytochemicals in the stem of *Skimmia laureola* was much significant.

Phyto chemical study of different fractions obtained from leaves of *Skimmia laureola*

The results obtained from different phytochemical tests of the *Skimmia laureola* leaves is shown in Table 6. The qualitative tests on the leaves discovered the existence of secondary metabolites in crude and other extracts under study, such as alkaloids, flavonoids, proteins, fats, oils, tannins, carbohydrates, sterols and saponins and glycosides. The samples were abundant in tannins, carbohydrates, sterols, proteins, flavonoids and lipids. The results obtained from the leaves of *Skimmia laureola* exposed the occurrence of proteins, carbohydrates and tannins in all the fractions under study whereas alkaloids, lipids and flavonoids were observed in all the extracts excluding aqueous extracted samples. However, sterols were found abundant in crude, butanol and aqueous extracts and were found absent in ethyl acetate, n-hexane fractions. The phytochemical analysis of the leaves extracts also demonstrated the moderate presence of saponins in crude, hexane and aqueous extracts of *Skimmia laureola* while showing negative results for butanol and ethyl acetate extracts.
respectively. Hence, the general presence of phytochemicals in the leaves of *Skimmia laureola* was significant.

**Insecticidal activity of several solvent extracts obtained from the roots of *Viola pilosa* against *Tribolium castaneum***

Figure 160 shows the results regarding the insecticidal potential achieved from the five solvents extracts from the roots of *Viola pilosa* plant against *Tribolium castaneum*. The primary analysis of *Viola pilosa* against the insect revealed that all the five solvent extracted fractions presented variable and significant insect and dose dependent insecticidal capability. The final data concluded that amongst all these samples under study, the maximum mortality rate of 30% was presented by ethyl acetate fraction at a concentration of 18µl disc\(^{-1}\) followed by hexane and crude samples showing a value of 20% at the same concentration of 18µl disc\(^{-1}\) when matched to the control, while hexane and butanol fractions did not show any mortality at 6µl disc\(^{-1}\). However, the aqueous fraction exposed significant activities at all the concentrations under study.

**Insecticidal activity of solvent extracted samples from the roots of *Viola pilosa* against *S. oryzae***

The outcomes achieved from the insecticidal potential from the five solvents fractions from the roots of *Viola pilosa* plant against *S. oryzae* are presented in Figure 161. The primary analysis of *Viola pilosa* against the insect under study revealed that all the five solvent extracted fractions presented variable and significant insect and dose dependent insecticidal capability. The final data concluded that amongst all these samples under study, the maximum mortality rate of 23.3% was shown by ethyl acetate and crude at a concentration of 18µl disc\(^{-1}\) followed by aqueous and hexane extract samples showing a value of 20% at the same concentration of 18µl disc\(^{-1}\) when compared to the control, while ethyl acetate, aqueous and butanol fractions did not show mortality at 6µl disc\(^{-1}\).
### Table 6: Different phytochemicals present in leaves of *Skimmia laureola*

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Crude</th>
<th>Butanol</th>
<th>Hexane</th>
<th>E.acetate</th>
<th>Aqueous</th>
</tr>
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<td>++</td>
<td>_</td>
</tr>
</tbody>
</table>

+++ : presence in abundance  
++  : presence in moderate quantity  
+   : presence but in less amount  
_   : complete absence of the compound

![Figure 160](image_url):

Insecticidal mortality (%), with standard deviation of various solvent extracted fractions from the roots of *Viola pilosa* against *Tribolium castaneum*.
Figure 161: Insecticidal mortality (%), with standard deviation of various solvent extracted fractions from the roots of *Viola pilosa* against *S. oryzae*.

Figure 162: Insecticidal mortality (%), with standard deviation of various solvent extracted fractions from the shoots of *Viola pilosa* against *Tribolium castaneum*. 
**Insecticidal activity of several solvent fractions obtained from the shoots of Viola pilosa against Tribolium castaneum**

Figure 162 shows the results regarding the insecticidal potential achieved from the five solvents extracts from the shoots of Viola pilosa plant against Tribolium castaneum. The primary analysis of Viola pilosa against the insect revealed that all the five solvent extracted fractions presented variable and significant insect and dose dependent insecticidal capability. The final data concluded that amongst all these samples under study, the maximum mortality rate of 30% was shown by crude at a concentration of 18µl disc$^{-1}$ followed by ethyl acetate extracted fractions showing a value of 23.3% at the same concentration of 18µl disc$^{-1}$ when compared to the control, while butanol and aqueous fractions did not show any mortality at 6µl disc$^{-1}$. However, the hexane sample presented significant activities at all the three concentrations under study.

**Insecticidal activity of solvent extracts obtained from the shoots of Viola pilosa against S. oryzae**

The outcomes achieved from the insecticidal potential from the five solvents extracts from the shoots of Viola pilosa plant against S. oryzae are presented in Figure 163. The primary analysis of Viola pilosa against the insect under study revealed that all the five solvent extracted fractions presented variable and significant insect and dose dependent insecticidal capability. The final data concluded that amongst all these samples under study, the maximum mortality rate of 23.3% was shown by hexane at a concentration of 18µl disc$^{-1}$ followed by crude extracted fractions showing a value of 20% at the same concentration of 18µl disc$^{-1}$ when compared to the control, while ethyl acetate, crude and aqueous fractions were not capable to express any mortality at 6µl disc$^{-1}$. However, the hexane and n-butanol fractions showed significant activities at all the concentrations under study.
**Figure 163:** Insecticidal mortality (%), with standard deviation of various solvent extracted fractions from the shoots of *Viola pilosa* against *S. oryzae*.

**Figure 164:** Insecticidal mortality (%), with standard deviation of various solvent extracted fractions from the stem of *Skimmia laureola* against *Tribolium castaneum.*
Insecticidal potential of various fractions from the stem of *Skimmia laureola* against *Tribolium castaneum*

Figure 164 shows the results regarding the insecticidal potential achieved from the five solvents extracts from the stem of *Skimmia laureola* plant against *Tribolium castaneum*. The primary analysis of *Skimmia laureola* against the insect revealed that all the five solvent extracted fractions presented variable and significant insect and dose dependent insecticidal capability. The final data concluded that amongst all these samples under study, the maximum mortality rate of 30% was shown by ethyl acetate extract at a concentration of 18µl disc$^{-1}$ followed by aqueous and hexane, showing a value of 23.3% at the same concentration of 18µl disc$^{-1}$ when compared to the control, while butanol and crude fractions did not show any mortality at 6µl disc$^{-1}$ and 12µl disc$^{-1}$. However, the butanol and aqueous extracts exposed significant activities at all the test concentrations under study.

Insecticidal activity of different plants extracts obtained from the stem of *Skimmia laureola* against *S. oryzae*.

The results achieved from the insecticidal potential from the five solvents extracts from the stem of *Skimmia laureola* against *S. oryzae* are presented in Figure 165. The primary analysis of *Skimmia laureola* against the insect under study revealed that all the five solvent extracted fractions presented variable and significant insect and dose dependent insecticidal capability. The final data concluded that amongst all these samples under study, the maximum mortality rate of 23.3% was presented by n-hexane and n- butanol at a concentration of 18µl disc$^{-1}$ followed by aqueous extracted fractions showing a value of 20% at the same concentration of 18µl disc$^{-1}$ when compared to the control, while crude, hexane and ethyl acetate fractions did not show any mortality at 6µl disc$^{-1}$. However, the fractions obtained from butanol and aqueous offered significant activities at the tested concentrations under study.
Figure 165: Insecticidal mortality (%), with standard deviation of various solvent extracted fractions from the stem of *Skimmia laureola* against *S. oryzae*.

Figure 166: Insecticidal mortality (%), with standard deviation of various solvent extracted fractions from the leaves of *Skimmia laureola* against *Tribolium castaneum*.
Insecticidal potential of various fractions obtained from the leaves of *Skimmia laureola* against *Tribolium castaneum*

Figure 166 shows the results regarding the insecticidal potential achieved from the five solvents extracts from the leaf samples of *Skimmia laureola* plant against *Tribolium castaneum*. The primary analysis of *Skimmia laureola* against the insect revealed that all the five solvent extracted fractions presented variable and significant insect and dose dependent insecticidal capability. The final data concluded that amongst all these samples under study, the maximum mortality rate of 23.3% was shown by hexane and aqueous at a concentration of 18µl disc⁻¹ followed by hexane and aqueous extracted fractions showing a value of 13.3% at the concentration of 12µl disc⁻¹ when compared to the control, while ethyl acetate, butanol and crude fractions did not show any mortality at 6µl disc⁻¹ and 12µl disc⁻¹. However, the aqueous fractioned samples disclosed significant activities at all the test concentrations under study.

Insecticidal strength of various fractions obtained from the leaves of *Skimmia laureola* against *S. oryzae*.

The results achieved from the insecticidal potential from the five solvents extracts from the leaves of *Skimmia laureola* against *S. oryzae* are presented in Figure 167. The primary analysis of *Skimmia laureola* against the insect under study revealed that all the five solvent extracted fractions presented variable and significant insect and dose dependent insecticidal capability. The final data concluded that amongst all these samples under study, the maximum mortality rate of 23.3% was shown by butanol at a concentration of 18µl disc⁻¹ followed by hexane and aqueous extracted fractions showing a value of 20% at the same concentration of 18µl disc⁻¹ when compared to the control, while crude, aqueous, ethyl acetate and hexane fractions did not show any mortality at 6µl disc⁻¹. However, the butanol fractioned extract unveiled significant activities at all the test concentrations under study.
**Figure 167:** Insecticidal mortality (%), with standard deviation of various solvent extracted fractions from the leaves of *Skimmia laureola* against *S. oryzae*.

**Figure 168:** Phytotoxicity level (%), with standard deviation values of various solvent extracted fractions from the roots of *Viola pilosa*. 
Phytotoxic activities of Viola pilosa and Skimmia laureola

The phytotoxic potential of five solvents extracted fractions from Viola pilosa and Skimmia laureola were also carried out by the help of Lemna minor plant for the verification of the phytotoxic activity of each extract.

Phytotoxic activities of varied solvent fractioned samples obtained from the roots of Viola pilosa

The data attained from the phytotoxic bioassay of solvent extracts from the root of Viola pilosa plant is illustrated in Figure 168. The analysis of phytotoxity on Viola pilosa root revealed a significant phytotoxic level for all the solvent extracts samples against Lemna minor. The statistical data specified that amongst these fractions, the highest level of phytotoxicity (32.2% at 18µl disc\(^{-1}\)) was showed by crude methanolic extract followed by ethyl acetate (28.86 % at 18µl disc\(^{-1}\)) when matched to remaining fractioned samples and control. The lowermost phytotoxic potential of 4.43% was noticed for the aqueous extracted fraction at a concentration of 6µl disc\(^{-1}\).

The results also declared that a significant phytotoxic inhibition level against lemna minor was showed by hexane, butanol, crude and ethyl acetate at all the three concentrations whereas the inhibition level of water extracted fraction was temperate. The sequence of the phytotoxic level of the five solvent extracted fractions was methanolic crude > ethyl acetate > n- butanol > n-hexane > aqueous.

Phytotoxic activities of solvent fractioned samples obtained from the shoots of Viola pilosa

The data attained from the phytotoxic bioassay of solvent extracts from the shoots of Viola pilosa plant is illustrated in Figure 169. The analysis of phytotoxicity on Viola pilosa shoots revealed a significant phytotoxic level for all the solvent extracts samples against Lemna minor. The statistical data specified that amongst these fractions, the highest level of phytotoxicity (47.76% at 18µl disc\(^{-1}\)) was showed by hexane extract followed by butanol (42.2 % at 18µl disc\(^{-1}\)) when matched to remaining fractioned samples and control. The bottommost phytotoxic potential of 14.43% was noticed for the aqueous and crude methanolic extracted fractions at a concentration of 6ul disc\(^{-1}\). The results also declared that a significant phytotoxic
inhibition level against *lemna minor* was showed by butanol, ethyl acetate, crude and n-hexane at all the three concentrations whereas the inhibition level of water extracted fraction was temperate. The sequence of the phytotoxic level of the five solvent extracted fractions was hexane > butanol > ethyl acetate > crude > aqueous.

**Phytotoxic activities of varied solvent fractioned samples obtained from the stem of *Skimmia laueola***

The data attained from the phytotoxic bioassay of solvent extracts from the stem of *Skimmia laueola* is illustrated in Figure 170. The analysis of phytotoxicity on *Skimmia laueola* stem revealed a significant phytotoxic level for all the solvent extracts samples against *Lemna minor*. The statistical data specified that amongst these fractions, the highest level of phytotoxicity (97.76% at 18µl disc⁻¹) was showed by aqueous extract followed by the same extract giving a value of 66.66% at a concentration of 12µl disc⁻¹ when compared to other extracts and control. The third highest value of toxicity (55.5% at 18µl disc⁻¹) was noticed for butanol extracted fraction. The lowest phytotoxic potential of 20% was noticed for the hexane fractioned sample at a concentration of 6µl disc⁻¹. The outcomes also declared that a significant phytotoxic inhibition level against *lemna minor* was showed by aqueous, butanol, ethyl acetate and crud at all the three concentrations whereas the inhibition level of hexane extracted fraction was temperate. The sequence of the phytotoxic level of the five solvent extracted fractions was methanolic aqueous > butanol > crude > ethyl acetate > n-hexane.

**Phytotoxic activities of solvent fractions obtained from the leaves of *skimmia laureola***

The data attained from the phytotoxic bioassay of solvent extracts from the leaves of *Skimmia laureola* plant is illustrated in Figure 171. The analysis of phytotoxicity on *Skimmia laureola* leaves revealed a significant phytotoxic level for all the solvent extracts samples against *Lemna minor*. The statistical data specified that amongst these fractions, the highest level of phytotoxicity (96.66% at 18µl disc⁻¹) was showed by hexane extract followed by the same extract giving a value of 86.66% at a concentration of 12µl disc⁻¹ when compared to other extracts and control. The third
highest value of toxicity (58.86% at 18µl disc⁻¹) was noticed for ethyl acetate extracted fraction. The lowest phytotoxic potential of 15.53% was noticed for the crude methanolic fraction at a concentration of 6µl disc⁻¹. The results also declared that a significant phytotoxic inhibition level against *lemna minor* was showed by aqueous, hexane, ethyl acetate and butanol at all the three concentrations whereas the inhibition level of methanolic crude fraction was temperate. The sequence of the phytotoxic level of the five solvent extracted fractions was aqueous > ethyl acetate > hexane > butanol > crude.
Figure 169: Phytotoxicity level (%), with standard deviation values of various solvent extracted fractions from the shoots of *Viola pilosa*.

Figure 170: Phytotoxicity level (%), with standard deviation values of various solvent extracted fractions from the stem of *skimmia laureola*.
Figure 171: Phytotoxicity level (%), with standard deviation values of various solvent extracted fractions from the leaves of *skimmia laureola*
V. DISCUSSION

Nanotechnology is a multi-disciplined field, which holds a diverse and vast range of devices resulting from biology, engineering, chemistry and physics. (Ferrari, 2005). Nanoparticles are considered as the basic building blocks of Nano materials (Swihart, 2003). There are several techniques for the creation and bulk production of the gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) (Narayan and Sakyhivel, 2011). Generally, four different methods like bio inspired or biogenic, physiochemical, physical and chemical are being used for the synthesis of the nanoparticles (that are mainly colloids). These methods have been sub classified to obtain the nanoparticles of various structures and sizes. Biogenic method of nanoparticles synthesis is considered as the most effective one because all the other methods involve use of harsh substances and chemicals, severe synthesis environments, large capital intensives, a reduced amount of production and high energy. As a result, these practices are environmentally non friendly and fierce whereas, the bio inspired approaches with less hazards, ecological synthesis, high production, less expenses, non-toxicity are naturally benign and responsive (Shedbalkar et al., 2014).

Various kinds of green or biogenic methods can be used for the synthesis of nanoparticles but the microorganism based techniques are well understood and considered as the most promising one (Logeswari et al., 2013). Several strains of microorganisms are being used for the green synthesis, however bacterial and fungal strains are found more effective in the synthesis of nanoparticles. All the approaches that utilize the different strains of fungi and bacteria are efficient in the metallic synthesis of nanoparticles. However, the net production is usually very low (Valentina and Minaev, 2014). Due to the numerous synthesis and environmental concerns, the current research was mainly designed to focus on the biogenic synthesis of nanoparticles by using the ecofriendly elements like silver nitrate gold chloride for a stable and high production. Environmentally benign protocols were optimized for the green or biogenic synthesis of gold and silver nanoparticles from the different extracts of plants (Ravindran et al., 2013, Khan et al., 2013, Ateeq et al., 2015).
For the characterization of nanoparticles, only a single characterization method is not suggested by the scientists, therefore in the present study a vast range of characterization techniques were adopted for the better understanding of various aspects of the nanoparticles. The UV–Vis spectroscopic technique is routinely used for the recognition of the newly synthesized metallic nanoparticles. The gold and silver nanoparticles spectroscopic peaks lie in the ranges of 500-600 nm and 350-450 nm respectively (Ocanas et al., 2010). The Fourier Transform Infrared Spectroscopic technique (FT-IR) is mainly used for the characterization of the different functional groups involved in the reduction and fabrication of gold and silver ions, during the synthesis of nanoparticles from the extracts of plant materials (Marco et al., 2010). For the determination of different phases, crystalline structure, nature and average size of the synthesize nanoparticles, a technique called XRD or X-ray diffraction is normally used (Harekrishna et al., 2009). To explicate the complete morphology and structure, a series of spectroscopic techniques like transmission electron microscopy (TEM), scanning electron microscopy (SEM), Field Emission Scanning Electron Microscopy (FESEM) and Atomic Force Microscopy (AFM) are available which help in complete imaging and mapping of the NPs. (Smetana et al., 2005; Ravindran et al., 2010; Ateeq et al., 2015). In the present study, we performed UV-Vis. Spectroscopy, SEM, XRD and FT-IR for the complete characterization of the AuNPs and AgNPs synthesized from the solvent extracted plants samples.

**Bio inspired green synthesis of gold nanoparticles (AuNPs)**

The methanolic crude and aqueous extracts solutions from the roots and shoots of *Viola pilosa* & *Skimmia laureola* leaves and stem were used for the synthesis of the biogenic AuNPs. (50mg plant extract / 100 ml de-ionized H2O) by the reduction of .1 mM of AuCl3 solution. The gold nanoparticles from the plant extracts were initially observed optically for the color change and were then confirmed by the help of UV-Vis Spectrophotometric technique. When methanolic crude and aqueous extracts solutions plant were treated and shaken with 0.1mM AuCl3 solution, the color of the resulted solution started changing from light yellow shade to dense pink and later on turned to deep purple (Annamalai et al., 2013). The change in color was so clear that it could be easily seen through a naked eye. The presence of deep purple color was a sign of synthesis of a good amount of gold nanoparticles. It was normally observed
that an increase in the ratio of combination of AuCl$_3$ solution and plant extracted fraction resulted in more dense colored solutions referring to the synthesis of a good amount of gold nanoparticles AuNPs. Specifically, it was observed that an increase in concentration of AuCl$_3$ solution in combination with the extracts resulted in a larger fractions at all the concentrations under study. The UV–vis spectroscopic technique was used for the confirmation of nanoparticles synthesis. There are free electrons in AuCl$_3$ that result in rising of SPR or Surface Plasma Resonance (Gosh and Pal 2007). The UV-Vis Spectrophotometer detects a resonance which is produced by the collective vibration of the gold nanoparticles and light waves and provides a characteristic peak in a range of 500nm-600nm thus showing the formation of nanoparticles (Kumar et al., 2011; Ravindran et al., 2010). For the complete characterization the AuNPs were subjected to SEM, XRD and FTIR analysis. The crystal structure and average size of AuNPs was determined through XRD. The presence of possible functional groups responsible for gold reduction was investigated by using FT-IR technique. Furthermore, the size and shape of the AuNPs were studied through SEM analysis technique. Temperature and salt stress was given to check the stability of the biogenic AuNPs.

**UV-Vis confirmation of gold nanoparticles (AuNPs)**

Formation of gold nanoparticles (AuNPs) by the combination of various ratios of 0.1mM AuCl$_3$, methanolic crude and aqueous extract solutions of roots and shoots of *Viola pilosa* and *Skimmia laureola* (50mg plant extract / 100 ml de-ionized H$_2$O) were confirmed by the help of UV-Vis spectrophotometer. The obtained spectrum specified that the highest peak was observed by the combination of 4ml of AuCl$_3$ solution and 1ml of the plant crude root extract (4:1). Final analysis of the curve for Viola root crude revealed an absorbance of 0.099 of the nanoparticles solution at the highest peak of 537.72nm wavelength. The optimum range for the AuNPs is between 500 nm - 600 nm. The wavelength of 537.72 nm comes within the required range hence confirming the formation of the gold nanoparticles. Other ratios resulted in either zero peaks or abnormal peaks. The final optimized extract ratios chosen for advance studies were 4:1 (4ml of AuCl$_3$ (0.1mM) and 1ml of plant extract) for *Viola* crude root extracts, *Viola* shoots crude and *Viola* shoots aqueous extracts respectively. However, 2:1 (2ml of AuCl$_3$ (0.1mM) and 1ml of plant extract) for the *Viola* root
aqueous extracts. Similarly, a ratio of 1:1 (1ml of AuCl$_3$ (0.1mM) and 1ml of plant extract) was observed for the *Skimmia* stem aqueous extract, a ratio of 4:1 (4ml of AuCl$_3$ (0.1mM) and 1ml of plant extract) was observed for stem crude and leaves aqueous extracts of *Skimmia laureola* and 3:1 (3ml of AuCl$_3$ (0.1mM) and 1ml of plant extract) was observed for the *Skimmia* leaves crude extract. The results indicated a separate color for each type of nanoparticles synthesized from crude methanolic and aqueous fractions of *Viola pilosa* & *Skimmia laureola*.

Comprehension of the spectra exposed that the nanoparticles solutions of 4:1 ratio showed absorbance of 0.099 at 537.72 nm, 0.496 at 531.76 nm, 0.362 at 548.81 nm, 0.797 at 536.20 nm and 0.099 at 556.91 nm wavelength for *Viola* crude root, *Viola* shoots crude, *Viola* shoots aqueous, *Skimmia* stem crude and *Skimmia* leaves aqueous extracted AuNPs respectively. As 537.72 nm, 531.76 nm and 548.81 nm fall within the range of 500 nm – 600 nm (Das et al., 2010), the formation of gold nanoparticles was confirmed. The smallest peak was recorded for the solution of 1ml AuCl$_3$ and 1ml of extract solution in *Skimmia* leaves aqueous extracted AuNPs. Generally, the nanoparticles samples with equivalent or higher quantities of AuCl$_3$ solution than the plant extracts showed synthesis of gold nanoparticles (2:1 and 3:1 ) whereas no synthesis was observed in all the tested ratios where the amount of plant extract was greater than AuCl$_3$ solution (1:5, 1ml AuCl$_3$ : 5ml extract solution).

**Heat stress stability analysis of AuNPs**

In the current study, the newly synthesized gold nanoparticles were subjected to stability tests like heat and salt concentrations (Malarkodi et al., 2013). The results showed that the stability of gold nanoparticles was inversely proportional to the increasing temperatures which might be due to the destruction of the active constituents loaded in the metallic particles (Paulkumar et al., 2013). The results also showed that the gold nanoparticles of *Viola pilosa* were found highly stable at the temperature range between 25°C and 50°C while the nanoparticles of *Skimmia* were stable at the temperature range between 20°C and 40°C. The study proved that an increase in temperature reduced the stability of the gold nanoparticles in a temperature ranging from 50°C and 60°C thus lowering the peak and stability of the tested samples. However, the further analysis reported that AuNPs were totally unstable and
were damaged at a temperature range of 60°C and 100°C presenting no absorbance through spectrophotometer. (Paulkumar et al., 2013).

**Salt stress stability analysis of AuNPs**

Sodium chloride (NaCl) was used as a standard salt to investigate the influence of salt stress on the gold nanoparticles of *Viola pilosa* and *Skimmia laureola*. The UV-Vis spectrophotometer was used for recording the results of the salt stress stability test. The results of experiment recommended that an inverse relationship was found between the concentration of Sodium chloride and degree of stability of nanoparticles. An increase in the NaCl concentration resulted in the reduced stability of the AuNPs. Moreover, it was also noticed that the molar (1M) concentration of NaCl adversely effected the AuNPs as compared to the milli molar concentrations. The AuNPs were found more stable at salt stress in milli-Molar concentrations. Gnanajobitha et al., 2013 also achieved similar effects while working with salts stress of the nanoparticles. The AuNPs were found more stable at a concentration of 0.1mM and decrease in stability was noticed upon increase in salt concentration. Amongst the tested concentrations, the nanoparticles were found much stable at 0.1mM, comparatively moderately stable at 0.5Mm and least stable at a salt concentration of 1M. (Noruzi et al., 2011).

**FT-IR spectra analysis of AuNPs**

The gold nanoparticles (AuNPs) synthesized from the crude methanolic and aqueous extracts of *Viola pilosa* and *Skimmia laureola* were subjected to Fourier Transformed Infrared (FTIR) spectroscopic analysis for the identification of possible functional groups responsible for reduction of gold. A particular mode of vibration is stimulated by the interaction of the molecules with a photon of a definite wavelength. This specific stimulation causes rise in the strength of vibration which results in a constructive interference showing a peak at the completion (Brandi, 2009). This constructive interference points to presence of various functional groups existing in the plant extracts nanoparticles by generating a specific spectrum (Genc et al., 2011).
FTIR spectra of *Viola* roots and shoots methanolic crude extract proposed that the roots and shoots extracted nanoparticles presented major absorption bands of infrared radiation at wave numbers 3315.6 cm\(^{-1}\), 3316.3 cm\(^{-1}\), 1639.7 cm\(^{-1}\), 1219.6 cm\(^{-1}\) and 1219.7 cm\(^{-1}\) respectively. It was discovered that the infrared radiation absorption band at wave numbers 3315.6 cm\(^{-1}\) and 3316.3 cm\(^{-1}\) present in spectra of nanoparticles presented Phenols. The shift in the band discovered that the –O–H functional group holding compounds were mainly responsible for the reduction of gold (Au) metal. It was noticed that absorption band at wave numbers 1639.7 cm\(^{-1}\), 1219.6 cm\(^{-1}\) and 1219.7 cm\(^{-1}\) in spectra of nanoparticles were found to be in the range of 1600-1750 and 1200-1300, thus presenting esters. Sharp Peaks at 1639.7 cm\(^{-1}\) represent carbonyl group of esters. (C═O) and corresponding peak at 1219.6 cm\(^{-1}\) and 1219.7 cm\(^{-1}\) represents ether group of esters. (R–O–R). (Noruzi et al., 2011)

FTIR spectra analysis of *Viola* roots and shoots aqueous extract proposed that the roots and shoots extracted nanoparticles presented major absorption bands of infrared radiation at wave numbers 3328.0 cm\(^{-1}\), 3300.6 cm\(^{-1}\) and 1639.7 cm\(^{-1}\) respectively. It was discovered that the infrared radiation absorption band at wave numbers 3328.0 cm\(^{-1}\) and 3300.6 cm\(^{-1}\) present in spectra of nanoparticles presented Phenols. The shift in the band discovered that the –O–H functional group holding compounds were mainly responsible for the reduction of gold (Au) metal. It was also noticed that absorption band at wave numbers 1639.7 cm\(^{-1}\) in spectra of nanoparticles were found to be in the range of 1600-1750 thus presenting esters. Sharp Peaks at 1639.7 cm\(^{-1}\) represent carbonyl group of esters. (C═O). These results are in accordance with (Zayed and Eisa, 2014) who stated that phenolic compounds are responsible for the synthesis of AuNPs.

FTIR spectra of *Skimmia* leaves aqueous extract proposed that the leaves extracted nanoparticles presented major absorption bands of infrared radiation at wave numbers 3301.6 cm\(^{-1}\), 1639.7 cm\(^{-1}\) and 1219.5 cm\(^{-1}\) respectively. The infrared radiation absorption band at wave number 3301.6 cm\(^{-1}\) present in spectra of nanoparticles were found to be Phenols. The shift in the band also discovered that the –O–H functional group holding compounds were mainly responsible for the reduction of gold (Au) metal. It was also noticed that absorption band at wave numbers 1639.7 cm\(^{-1}\) and 1219.5 cm\(^{-1}\) in spectra of nanoparticles were found to be in the range of
1600-1750 and 1200-1300, thus presenting esters. Sharp Peaks at 1639.7 cm\(^{-1}\) represent carbonyl group of esters. (C = O) and corresponding peak at 1219.5 represents ether group of esters. (R–O–R). Similar results of functional groups participation in synthesis of AuNPs were reported by (Elavazhagan and arunachalam, 2011).

FTIR spectra analysis of *Skimmia* stem crude, aqueous and *Skimmia* leaves crude proposed that the stem and leaves extracted nanoparticles presented major absorption bands of infrared radiation at wave numbers 3323.8 cm\(^{-1}\), 3316.0 cm\(^{-1}\), 3315.1 cm\(^{-1}\), 1639.7 cm\(^{-1}\) and 1639.8 cm\(^{-1}\) respectively. The absorption band at wave numbers 3323.8 cm\(^{-1}\), 3316.0 cm\(^{-1}\) and 3315.1 cm\(^{-1}\) presented Phenols. The shift in the band discovered that the –O–H functional group holding compounds were mainly responsible for the reduction of gold (Au) metal. It was also noticed that absorption band at wave numbers 1639.7 cm\(^{-1}\) and 1639.8 cm\(^{-1}\) in spectra of nanoparticles were found to be in the range of 1600-1750 thus presenting esters. Sharp Peaks at 1639.7 cm\(^{-1}\) and 1639.8 cm\(^{-1}\) represent carbonyl group of esters. (C=O). Song et al. (2009) reported the involvement of phenolic compounds for the synthesis of AuNPs.

**X-Ray Diffraction (XRD) study of AuNPs**

The demonstrative X-Ray diffraction pattern of the powdered gold nanoparticles of *Viola* shoot crude indicated three distinctive deflection peaks within the 2 theta range at angles of 13.07°, 27.76° and 38.32° respectively, acting to be indexed in the diverse planes (110), (310) and (312) of the facets of gold nanoparticles. The XRD study carried out for the evaluation of the crystalline configuration of the gold nanoparticles produced from the aqueous extract of *Viola* shoots specified distinctive peaks at two theta values of 28.41°, 37.99° and 40.55° parallel to (100), (101) and (110) surfaces of gold NPs respectively. The index of Bragg’s reflections was done on the source of face centered cubic Au structure. The dimension of the facets revealed the crystal centrosymmetric nature of the synthesized gold nanoparticles. XRD patterns of gold nanoparticles were also verified by (Ghodake et al., 2010) and (Shankar et al., 2003). The X-Ray diffraction conformation of the powdered gold nanoparticles of *Viola* roots crude indicated four distinctive deflection peaks within the 2 theta range at angles of 8.59°, 28.08°, 38.32°,
64.84° and 77.94° respectively, acting to be indexed in the diverse planes (002), (106), (107), (205), (306) and (400) of the facets of gold nanoparticles. Examination of X-Ray diffraction peaks of gold nanoparticles produced from the aqueous extract of Viola roots specified distinctive peaks at two theta values of 22.02°, 28.39°, 37.99°, 44.37°, 64.82° and 77.94° parallel to (110), (111), (112), (200), (220) and (311) surfaces of gold NPs respectively. The demonstrative X-Ray diffraction pattern of the powdered gold nanoparticles of Skimmia stem crude indicated three distinctive deflection peaks within the 2 theta range at angles of 32.56°, 37.99°, 44.70°, 64.84° and 77.61° respectively, acting to be indexed in the diverse planes (101), (111), (200), (220), and (311) of the facets of silver nanoparticles. The XRD study carried out for the evaluation of the crystalline configuration of the gold nanoparticles produced from the aqueous extract of skimmia stem specified distinctive peaks at two theta values of 38.01°, 44.71°, 64.80° and 77.59° parallel to (111), (200), (220) and (311) surfaces of gold NPs respectively. (Jayaseelan et al., 2013) and Nagaraj et al., 2014) described the matching Bragg’s reflections. The X-Ray diffraction confirmation of the powdered gold nanoparticles of Skimmia leaves crude indicated four distinctive deflection peaks within the 2 theta range at angles of 15.95°, 26.80°, 38.63°, and 45.66° respectively, acting to be indexed in the diverse planes (110), (111), (112) and (120) of the facets of gold nanoparticles. The consequences of the XRD study carried out for the evaluation of the crystalline configuration of the gold nanoparticles produced from the aqueous extract of Skimmia leaves specified distinctive peaks at two theta values of 13.33°, 25.12° and 38.48° parallel to (011), (111) and (123) surfaces of gold NPs respectively.

The dimension of the facets of all the parts of Viola pilosa and Skimmia laureola revealed the crystalline centrosymmetric nature of the synthesized gold nanoparticles. The full width half maximum determination of the most intense peak, by the help of Sherrer equation was used for the typical size calculation of the synthesized AuNPs, which appeared as 3.65 nm, 16.82 nm, 27.12 nm, 26.43 nm, 9.91 nm, 10.76 nm, 20.94 nm and 21.29 nm for Viola root crude AuNPs, Viola root aqueous AuNPs, Viola shoot crude AuNPs, Viola shoot aqueous AuNPs, Skimmia stem crude, Skimmia stem aqueous AuNPs, Skimmia leaves crude and Skimmia leaves
aqueous AuNPs respectively. The sharpness of the peak undoubtedly specified that the nanoparticles were present in nano region. (Singaravelu et al., 2007) and (Aromal and Philip, 2012) also did analysis on XRD of AuNPs. The results also declared no peaks were recorded for the XRD patterns because of the crystallographic impurities. It means that the biogenic gold nanoparticles were extremely pure in nature (Pathipati and Rajasekharreddy, 2011).

**Size determination of AuNPs by SEM**

SEM technique was carried out to determine the morphological characters like shape and size of gold nanoparticles synthesized from methanolic crude and aqueous extracts of *Viola pilosa* and *Skimmia laureola*. The results of SEM carried out on of AuNPs produced from plants extracts showed average sizes of 20 nm, 25 nm, 20 nm, 50 nm, 50 nm, 50 nm, 20 nm and 50 nm for *Viola* root crude AuNPs, *Viola* root aqueous AuNPs, *Viola* shoot crude AuNPs, *Viola* shoot aqueous AuNPs, *Skimmia* stem crude, *Skimmia* stem aqueous AuNPs, *Skimmia* leaves crude and *Skimmia* leaves aqueous AuNPs respectively. The study also proposed that all the biogenic AuNPs of plant extracts were of uniform in morphology and were almost spherical in shape. (Leela and Viveknandan, 2008; Naveena and Prakash, 2013).

**Bio inspired green synthesis of silver nanoparticles (AgNPs)**

The methanolic crude and aqueous extracts solutions from the roots and shoots of *Viola pilosa* & *Skimmia laureola* leaves and stem were used for the synthesis of the biogenic AgNPs (50mg plant extract / 100 ml de-ionized H₂O) by the reduction of .1 mM of silver nitrate solution. (Amin et al., 2012). The silver nanoparticles from the plant extracts were initially observed optically for the color change and were then confirmed by the help of UV-Vis Spectrophotometric technique. When methanolic crude and aqueous extracts solutions plant were treated and shaken with 0.1mM silver nitrate solution, the color of the resulted solution started changing from light yellow shade to dense yellow and later on turned to deep brown (Annamalai et al., 2013). The change in color was so clear that it could be easily seen through a naked eye. The presence of deep brown color was a sign of synthesis of a good amount of silver nanoparticles. It was normally observed that an increase in the ratio of combination of
AgNO₃ solution and plant extracted fraction resulted in more dense colored solutions referring to the synthesis of a good amount of silver nanoparticles AgNPs. (Bar et al., 2009a) and (Pantelis et al., 2012) also observed the change in colour from light yellow to dense confirming the formation of nanoparticles. The UV–vis spectroscopic technique was used for the confirmation of nanoparticles synthesis. There are free electrons in AgNO₃ that result in rising of SPR or Surface Plasma Resonance (Gosh and Pal, 2007). The UV-Vis Spectrophotometer detects a resonance which is produced by the collective vibration of the silver nanoparticles and light waves and provides a characteristic peak in a range of 350nm-450nm thus showing the formation of nanoparticles. (Umoren et al., 2014). For the complete characterization, the AgNPs were subjected to SEM, XRD and FTIR analysis. The crystal structure and average size of AgNPs was determined through XRD. The presence of possible functional groups responsible for silver reduction was investigated by using FT-IR technique. Furthermore, the size and shape of the AuNPs were studied through SEM analysis technique. Temperature and salt stress was given to check the stability of the biogenic AgNPs.

**UV-Vis confirmation of silver nanoparticles (AgNPs)**

Formation of silver nanoparticles (AgNPs) by the combination of various ratios of 0.1mM AgNO₃, methanolic crude and aqueous extract solutions of roots and shoots of *Viola pilosa* and *Skimmia laureola* (50mg plant extract / 100 ml de-ionized H₂O) were confirmed by the help of UV-Vis spectrophotometer (Bar et al., 2009b). The obtained spectrum specified that the highest peak was observed by the combination of 1ml of AgNO₃ solution and 10ml of the *Viola* shoot crude, *Viola* shoot aqueous and *Skimmia* leaves crude extract (1:10). Final analysis of the curve for *Viola* shoot crude, *Viola* shoot aqueous and *Skimmia* leaves crude extract revealed an absorbance of 1.993, 1.994 and 2.493 of the nanoparticles solution at the highest peaks of 400.93 nm, 395.66 nm and 421.36 nm wavelengths respectively. The optimum range for the AgNPs is between 350 nm - 450 nm. The wavelengths of 400.93 nm, 395.66 nm and 421.36 nm fall within the required range hence confirming the formation of the silver nanoparticles. (Song and Kim, 2009). Other ratios resulted in either zero peaks or abnormal peaks. The final optimized extract ratios chosen for
advance studies were 1:5 (1ml of AgNO₃ (0.1mM) and 5ml of plant extract) for Viola root crude extracts, Viola roots aqueous, Skimmia stem aqueous, Skimmia stem crude and Skimmia leaves aqueous extracts respectively. However, 1:10 (1ml of AgNO3 (0.1mM) and 10ml of plant extract) for the Viola shoot crude, Viola shoot aqueous and Skimmia leaves crude extracts. The results indicated a separate color for each type of nanoparticles synthesized from crude methanolic and aqueous fractions of Viola pilosa & Skimmia laureola.

Comprehension of the spectra exposed that the nanoparticles solutions of 1:5 ratio showed absorbance of 1.487 at 415.04 nm, 1.600 at 380.70 nm, 2.989 at 395.0 nm, 2.989 at 381.99 nm and 1.981 at 401.59 nm wavelength for Viola root crude extracts, Viola roots aqueous, Skimmia stem aqueous, Skimmia stem crude and Skimmia leaves aqueous extracted AgNPs respectively. As 415.04 nm, 380.70 nm, 395.0 nm, 381.99 nm and 401.59 nm fall within the range of 350 nm – 450 nm, the formation of silver nanoparticles was confirmed. Generally, the nanoparticles samples with equivalent or higher quantities of plant extracts than the AgNO₃ solution showed synthesis of silver nanoparticles (5:1 and 10:1) whereas no synthesis was observed in all the tested ratios where the amount of AgNO₃ solution was greater than the plant extract (5:1, 5ml AgNO₃ : 1ml extract solution).

**Heat stress stability analysis of AgNPs**

The temperatures of the any reaction play an important role in the biogenic synthesis of nanoparticles by regulating the nucleation speed for alignment of the nanoparticles (Bashir et al., 2013). Usually, the rate of reaction increases with an increase in the temperature range due to the magnification of molecular kinetic energy resulting in the boosted collision of molecules. This rise in the rate of reaction moves up to a certain level. Exceeding the certain limit results in the destruction of the components of the mixture (Vijay et al., 2014). In the current study, the newly synthesized silver nanoparticles were subjected to stability tests like heat, and salt concentrations. The results showed that the stability of silver nanoparticles was inversely proportional to the increasing temperatures which might be due to the destruction of the active constituents loaded in the metallic particles. The results also showed that the silver nanoparticles of Viola were found highly stable at the
temperature range between 25°C and 50°C while the nanoparticles of *Skimmia* were stable at the temperature range between 20°C and 40°C. Ganesan et al. (2013) described the best biogenic synthesis of the AgNPs in the range of 20°C - 50°C. The study proved that an increase in temperature reduced the stability of the silver nanoparticles in a temperature ranging from 50°C and 60°C thus lowering the peak and stability of the tested samples. However, the further analysis reported that AgNPs were totally unstable and were damaged at a temperature range of 60°C and 100°C presenting no absorbance through spectrophotometer. Raveendran et al. (2003) and Bashir et al. (2013) which confirmed the reversibility of AgNPs at higher temperature.

**Salt stress stability analysis of AgNPs**

Sodium chloride (NaCl) was used as a standard salt to investigate the influence of salt stress on the silver nanoparticles of *Viola pilosa* and *Skimmia laureola*. The UV-Vis spectrophotometer was used for recording the results of the salt stress stability test. The results of experiment recommended that an inverse relationship was found between the concentration of Sodium chloride and degree of stability of nanoparticles. An increase in the NaCl concentration resulted in the reduced stability of the AuNPs. Moreover, it was also noticed that the molar (1M) concentration of NaCl adversely effected the AgNPs as compared to the milli molar concentrations. The AgNPs were found more stable at salt stress in milli-Molar concentrations. Gnanajobitha et al. (2013) also achieved similar effects while working with salts stress of the gold nanoparticles. The AgNPs were found more stable at a concentration of 0.1 mM and decrease in stability was noticed upon increase in salt concentration. Amongst the tested concentrations, the nanoparticles were found much stable at 0.1 mM, comparatively moderately stable at 0.5 mM and least stable at a salt concentration of 1M. Noruzi et al. (2011) also found nanoparticles show decrease in stability upon increase in salt concentration.

**FT-IR spectra analysis of AgNPs**

The silver nanoparticles (AgNPs) synthesized from the crude methanolic and aqueous extracts of *Viola pilosa* and *Skimmia laureola* were subjected to Fourier Transformed Infrared (FT-IR) spectroscopic analysis for the identification of possible
functional groups responsible for reduction of silver. A particular mode of vibration is stimulated by the interaction of the molecules with a photon of a definite wavelength. This specific stimulation causes rise in the strength of vibration which results in a constructive interference showing a peak at the completion (Brandi, 2009). This constructive interference points to presence of various functional groups existing in the plant extracts nanoparticles by generating a specific spectrum (Genc et al., 2011).

FT-IR spectra of *Viola* roots crude, *Viola* shoots methanolic crude and *Viola* shoots aqueous extract proposed that the roots and shoots extracted nanoparticles presented major absorption bands of infrared radiation at wave numbers 3320.0 cm\(^{-1}\), 3316.3 cm\(^{-1}\), 3300.9 cm\(^{-1}\), 1639.8 cm\(^{-1}\), 1639.6 cm\(^{-1}\) and 1219.6 cm\(^{-1}\) respectively. By comparing the FT-IR spectrum of AuNPs to the FT-IR table values, it was discovered that the infrared radiation absorption band at wave numbers 3320.0 cm\(^{-1}\), 3316.3 cm\(^{-1}\) and 3300.9 cm\(^{-1}\) present in spectra of nanoparticles were found to be in the range of 3200-3550 when compared to the typical infrared absorption frequencies, thus presenting Phenols. The shift in the band discovered that the –O–H functional group holding compounds were mainly responsible for the reduction of silver (Ag) metal. It was also noticed that absorption band at wave numbers 1639.8 cm\(^{-1}\), 1639.6 cm\(^{-1}\) and 1219.6 cm\(^{-1}\) in spectra of nanoparticles were found to be in the range of 1600-1750 and 1200-1300, thus presenting esters. Sharp Peaks at 1639.7 cm\(^{-1}\) and 1639.6 cm\(^{-1}\) represent carbonyl group of esters. \((C = O)\) and corresponding peak at 1219.6 cm\(^{-1}\) represents ether group of esters. \((R – O – R)\). (Rajasekharreddy et al., 2010).

FT-IR spectra analysis of *Viola* roots aqueous extract proposed that the roots extracted nanoparticles presented major absorption bands of infrared radiation at wave numbers 3319.2 cm\(^{-1}\), and 1639.6 cm\(^{-1}\) respectively. The absorption band at wave numbers 3319.2 cm\(^{-1}\) showed Phenols. The shift in the band discovered that the –O–H functional group holding compounds were mainly responsible for the reduction of silver (Ag) metal. It was also noticed that absorption band at wave numbers 1639.6 cm\(^{-1}\) in spectra of nanoparticles were found to be in the range of 1600-1750 thus presenting esters. Sharp Peaks at 1639.6 cm\(^{-1}\) represent carbonyl group of esters. \((C = O)\). Vivek et al. (2011) stated that esters are responsible for the synthesis of AgNPs.
FT-IR spectra of *Skimmia* stem aqueous and *Skimmia* leaves aqueous extracts proposed that the stem and leaves extracted nanoparticles presented major absorption bands of infrared radiation at wave numbers 3304.8 cm\(^{-1}\), 3316.3 cm\(^{-1}\), 1639.8 cm\(^{-1}\) and 1219.6 cm\(^{-1}\) respectively. The infrared radiation absorption band at wave number 3304.8 cm\(^{-1}\) and 3316.3 cm\(^{-1}\) present in spectra of nanoparticles were found to be Phenols. The shift in the band discovered that the –O––H functional group holding compounds were mainly responsible for the reduction of silver (Ag) metal. It was also noticed that absorption band at wave numbers 1639.8 cm\(^{-1}\) and 1219.6 cm\(^{-1}\) in spectra of nanoparticles were found to be in the range of 1600-1750 and 1200-1300, thus presenting esters. Sharp Peaks at 1639.8 cm\(^{-1}\) represent carbonyl group of esters. (C═O) and corresponding peak at 1219.6 represents ether group of esters. (R––O–R). Ahmed et al. (2015) and Seeram et al. (2001) found similar results of functional groups participation in synthesis of AgNPs.

FT-IR spectra analysis of *Skimmia* stem crude and *Skimmia* leaves crude proposed that the stem and leaves extracted nanoparticles presented major absorption bands of infrared radiation at wave numbers 3315.8 cm\(^{-1}\), 3320.0 cm\(^{-1}\) and 1639.8 cm\(^{-1}\) respectively. It was discovered that the infrared radiation absorption band at wave numbers 3315.8 cm\(^{-1}\) and 3320.0 cm\(^{-1}\) present in spectra of nanoparticles were found to be in the range of 3200-3550 thus presenting Phenols. The shift in the band discovered that the –O–H functional group holding compounds were mainly responsible for the reduction of silver (Ag) metal. It was also noticed that absorption band at wave numbers 1639.8 cm\(^{-1}\) in spectra of nanoparticles were found to be in the range of 1600-1750 thus presenting esters. Sharp peaks at 1639.8 cm\(^{-1}\) represent carbonyl group of esters (C═O). These results are in accordance with Song et al. (2009) who reported the involvement of phenolic compounds for the synthesis of NPs.

**X-Ray Diffraction (XRD) study of AgNPs**

The demonstrative X-Ray diffraction pattern of the powdered silver nanoparticles of *Viola* shoot crude indicated three distinctive deflection peaks within the 2 theta range at angles of 13.39°, 29.68° and 38.32° respectively, acting to be indexed in the diverse planes (110), (301) and (411) of the facets of silver.
nanoparticles. The XRD study carried out for the evaluation of the crystalline configuration of the silver nanoparticles produced from the aqueous extract of Viola shoots specified distinctive peaks at two theta values of 27.76°, 29.68°, 32.56° and 37.67° parallel to (203), (212), (220) and (222) surfaces of silver NPs respectively. The index of Bragg’s reflections was done on the source of face centered cubic Ag structure. The dimension of the facets revealed the crystal centrosymmetric nature of the synthesized silver nanoparticles as showed and published by Chung et al. (2016). The X-Ray diffraction conformation of the powdered silver nanoparticles of Viola roots crude indicated four distinctive deflection peaks within the 2 theta range at angles of 23.94°, 32.23° and 38.32° respectively, acting to be indexed in the diverse planes (100), (211) and (222) of the facets of silver nanoparticles. Examination of X-Ray diffraction peaks of silver nanoparticles produced from the aqueous extract of Viola roots specified distinctive peaks at two theta values of 38.32°, 44.39° and 64.84° parallel to (111), (200) and (220) surfaces of silver NPs respectively.

The demonstrative X-Ray diffraction pattern of the powdered silver nanoparticles of Skimmia stem crude indicated three distinctive deflection peaks within the 2 theta range at angles of 32.89°, 38.95° and 77.94° respectively, acting to be indexed in the diverse planes (102), (104), and (130) of the facets of silver nanoparticles. The XRD study carried out for the evaluation of the crystalline configuration of the silver nanoparticles produced from the aqueous extract of Skimmia stem specified distinctive peaks at two theta values of 17.87°, 38.63°, 44.06° and 64.84° parallel to (111), (107), (110) and (119) surfaces of silver NPs respectively as previously published by Jayaseelan et al. (2013), Narayanan et al. (2010) and Nagaraj et al. (2014) describing the matching Bragg’s reflections. The X-Ray diffraction confirmation of the powdered silver nanoparticles of Skimmia leaves crude indicated five distinctive deflection peaks within the 2 theta range at angles of 19.62°, 29.58°, 32.19°, 38.33° and 63.35° respectively, acting to be indexed in the diverse planes (111), (222), (310), (400) and (541) of the facets of silver nanoparticles. The consequences of the XRD study carried out for the evaluation of the crystalline configuration of the silver nanoparticles produced from the aqueous extract of Skimmia leaves specified distinctive peaks at two theta values of 25.53°, 38.63° and 65.48° parallel to (100), (110) and (211) surfaces of silver NPs respectively.
The dimension of the facets of all the parts of *Viola pilosa* and *Skimmia laureola* revealed the spherical and cubic nature of the synthesized silver nanoparticles. The full width half maximum determination of the most intense peak, by the help of Sherrer equation was used for the typical size calculation of the synthesized AgNPs, which appeared as 3.48 nm, 17.93 nm, 19.31 nm, 18.50 nm, 5.56 nm, 10.32 nm, 9.50 nm and 11.51 nm for *Viola* root crude AgNPs, *Viola* root aqueous AgNPs, *Viola* shoot crude AgNPs, *Viola* shoot aqueous AgNPs, *Skimmia* stem crude, *Skimmia* stem aqueous AgNPs, *Skimmia* leaves crude and *Skimmia* leaves aqueous AgNPs respectively. The sharpness of the peak undoubtedly specified that the nanoparticles were present in nano region. The results also declared no peaks were recorded for the XRD patterns because of the crystallographic impurities. It means that the biogenic silver nanoparticles were extremely pure in nature. Zargar et al. (2011) also did analysis on XRD of AgNPs.

**Size determination of AgNPs by SEM**

SEM technique was carried out to determine the morphological characters like shape and size of silver nanoparticles synthesized from methanolic crude and aqueous extracts of *Viola pilosa* and *Skimmia laureola*. The results of SEM carried out on of AgNPs produced from plants extracts showed average sizes of 20 nm, 20 nm, 20 nm, 25 nm, 20 nm, 50 nm, 80 nm and 50 nm for *Viola* root crude AgNPs, *Viola* root aqueous AgNPs, *Viola* shoot crude AgNPs, *Viola* shoot aqueous AgNPs, *Skimmia* stem crude and *Skimmia* stem aqueous AgNPs, *Skimmia* leaves crude and *Skimmia* leaves aqueous AgNPs respectively. The study also proposed that all the biogenic AgNPs of plant extracts were of uniform in morphology and were almost spherical in shape. Dubey et al. (2009), Kumar et al. (2016) and Vijayakumar. (2013) also reported the uniform synthesis of the green synthesized nanoparticle from different plant extracts.

**Antibacterial activity of gold and nanoparticles (AuNPs and AgNPs) and various extracts of Viola pilosa and Skimmia laureola.**

The current study was also designed to investigate the antibacterial activities of different solvent extracted samples and their respective gold and silver
nanoparticles (AuNPs and AgNPs) from the roots and shoots of *Viola pilosa* and stem and leaves *Skimmia laureola*. Six different bacterial (gram positive and gram negative) strains were tested against all these extracts. Three different concentrations of 0.5 mg, 1 mg and 2 mg were selected for testing the microbes against plants extracts and their AuNPs and AgNPs. The antibacterial activities were determined through disc diffusion assay whereas antifungal potential was estimated using well diffusion method.

The statistical results regarding the antibacterial activities of the AuNPs of methanol extracted crude samples from the roots of *Viola pilosa* discovered that, in case of *Viola* root crude gold nanoparticles, the highest zone of inhibition was showed by *P. aeruginosa* at a concentration of 18µl disc\(^{-1}\) while the minimum inhibition zone was noticed for *K. pnemoniae* at a 6µl disc\(^{-1}\) concentration. The statistical analysis also proposed that *S. aureus*, *Xanthomonas* and *E. coli* presented good sensitivity to AuNPs, whereas, *K. pnemoniae* and *B. subtilis* exhibited moderate sensitivity to the nanoparticles. The statistical outcomes of the antibacterial activities of the AuNPs of the aqueous extracted fractions from the roots of *Viola pilosa* revealed the highest zone of inhibition by *P. aeruginosa* at a concentration of 18µl disc\(^{-1}\). The lowest zone of inhibition was noticed for *K. pnemoniae*. The statistical results further suggested that *E. coli*, *Xanthomonas*, *P. aeruginosa*, *B. subtilis* and *S. aureus* showed significant inhibition while *K. pnemoniae* showed less sensitivity to the water extracted AuNPs at all the three concentrations under study. Experimental results discovered in case of *Viola* shoot crude gold nanoparticles showed that the highest zone of inhibition was exposed by *P. aeroginosa* at a concentration of 18µl disc\(^{-1}\) while the minimum inhibition zone was noticed for *K. pnemoniae* at a 6µl disc\(^{-1}\) concentration. The statistical analysis also proposed that *Xanthomonas*, *E. coli* and *S. aureus* presented maximum sensitivity to AgNPs, whereas, *K. pnemoniae* and *B. subtilis* exhibited moderate sensitivity to the nanoparticles. The outcomes of the antibacterial activities of the AuNPs of the aqueous extracted fractions from the roots of *Viola pilosa* revealed that the highest zone of inhibition was presented by *P. aeruginosa* at a concentration of 18µl disc\(^{-1}\) while the lowest zone of inhibition was noticed for *K. pnemoniae*. The statistical results further suggested that *E. coli*, *Xanthomonas* and *S. aureus* showed significant inhibition while *K. pnemoniae* and *B. subtilis* showed less sensitivity to the water extracted AuNPs at all the three concentrations.
The statistical consequences about the antibacterial potential of the AuNPs prepared from the methanolic crude samples from the stem of *Skimmia Laureola* revealed that in case of *Skimmia* stem crude gold nanoparticles, the topmost zone of inhibition was displayed by *P. aeruginosa* at a concentration of 18µl disc⁻¹. However, the lowest inhibitory zone was noticed for *K.pnemoniae* and *B.subtilis* at a 6µl disc⁻¹ concentration. The statistical study also recommended that *E. coli*, and *S.aureus* presented extreme sensitivity to AgNPs, but *B.subtilis* and *K. pnemoniae* showed moderate sensitivity to the gold nanoparticles. (Jagtap and Bapat, 2013). The study discovered that in case of *Skimmia Laureola* stem aqueous gold nanoparticles, the maximum value of inhibition was revealed by *P. aeruginosa*, at a concentration of 18µl disc⁻¹. The lowest inhibitory zone value was noticed for *K.pnemoniae*. The statistical outcomes revealed that in case of *Skimmia* leaves crude gold nanoparticles, the topmost zone of inhibition was displayed by *P. aeruginosa* at a concentration of 18µl disc⁻¹ and the lowest inhibitory zone was noticed for *Xanthomonas* at a 6µl disc⁻¹ concentration. The statistical study also recommended that *E. coli* presented extreme sensitivity to AgNPs, but *K.pnemoniae* showed moderate sensitivity to the gold nanoparticles. In case of *Skimmia Laureola* leaves aqueous gold nanoparticles, the maximum value of inhibition was revealed by *P. aeruginosa*, at a concentration of 18µl disc⁻¹. The lowest inhibitory zone value was noticed for *K. pnemoniae*. The statistical results further suggested that *E.coli* and *Xanthomonas* showed good response against the AgNPs, while *K. pnemoniae* and *B. subtilis* showed less sensitivity to the water extracted AuNPs at all the three concentrations under study.

The statistical results regarding the antibacterial activities of the AgNPs of methanol extracted crude samples from the roots of *Viola pilosa* discovered that in case of *Viola* root crude silver nanoparticles, the highest zone of inhibition was showed by *P. aeruginosa* at a concentration of 18µl disc⁻¹. While the minimum inhibition zone was noticed for *Xanthomonas* at a 6µl disc⁻¹ concentration. In case of *Viola* root aqueous silver nanoparticles, the highest zone of inhibition was showed by *Xanthomonas* at a concentration of 18µl disc⁻¹. The statistical results further suggested that *P. aeruginosa*, *S.aureus*, *B. subtilis* and *K.pnemoniae* were proved to be fully resistant to the water extracted AgNPs at all the three concentrations under study. In case of *Viola* shoot crude silver nanoparticles, the highest zone of inhibition was
showed by *P. aeruginosa* at a concentration of 18µl disc⁻¹, while the minimum inhibition zone was noticed for *K. pnemoniae* at a 6µl disc⁻¹ concentration. The statistical results regarding the antibacterial activities of the AgNPs of methanol extracted crude samples from the shoots of Viola pilosa discovered that in case of Viola shoot crude silver nanoparticles, the highest zone of inhibition was showed by *P. aeruginosa* at a concentration of 18µl disc⁻¹. While the minimum inhibition zone was noticed for *K. pnemoniae* at a 6µl disc⁻¹ concentration.

The experimental outcomes revealed that in case of Skimmia stem crude silver nanoparticles, the topmost zone of inhibition was displayed by *P. aeruginosa* at a concentration of 18µl disc⁻¹. Whereas, the lowest inhibitory zone was noticed for *K. pnemoniae* at a 6µl disc⁻¹ concentration. In case of Skimmia Laureola stem aqueous silver nanoparticles, the maximum value of inhibition was revealed by *P. aeruginosa*, at a concentration of 18µl disc⁻¹. On the other hand *S. aureus* and *K. pnemoniae* showed complete resistance to the water extracted AgNPs at all the three concentrations under study. The statistical consequences about the antibacterial potential of the AgNPs prepared from the methanolic crude samples from the leaves of Skimmia Laureola revealed that the topmost zone of inhibition was displayed by *P. aeruginosa* at a concentration of 18µl disc⁻¹. However, the lowest inhibitory zone was noticed for *K. pnemoniae* and *B. subtilis* at a 6µl disc⁻¹ concentration. In case of Skimmia Laureola leaves aqueous silver nanoparticles, the maximum value of inhibition was revealed by *P. aeruginosa* at a concentration of 18µl disc⁻¹. The results also stated that *S. aureus* and *K. pnemoniae* showed complete resistance to the water extracted AgNPs at all the three concentrations under study. Similarly Ameer et al. (2009), Mendez et al.(2011), Prasad et al.(2011), Sarvesh et al.(2013), Malarkodi et al.(2013), Logeswari et al.(2013), Nagaraj et al. (2014), Thirumurguan et al. (2009) and Kumar et al. (2011) also reported anti-microbial activity of AgNPs against fungus and bacteria.

**Antibacterial activity of plant extracts against *P. aeruginosa***

*P. aeruginosa* (gram negative) bacterium is a noteworthy infectious agent of several diseases in human beings including pneumonia, malignant external otitis and meningitis (Bodey et al., 1983). The results of illustrate the antibacterial activity of
Viola pilosa root extracts against *P. aeruginosa*. The data attained from disc diffusion assay exposed that different solvent extracted samples measured varying degree of growth inhibition at all the tested concentrations. The results revealed that growth reduction of the tested microbe was dose dependent. Maximum growth inhibition was noted for ethyl acetate extracted samples at 2 mg disc\(^{-1}\) concentration followed by butanol at the same concentration. Similarly, minimum activity against the same microbe was measured by aqueous extracted samples at 0.5 mg disc\(^{-1}\) compared with other samples and controls. These results agree with Banaszczak et al. (2005), Arora et al. (2007), Fazal et al. (2012) and Pranting et al. (2010) who reported that crude methanolic extracted samples from *Viola tricolor* and *Viola odorata* effectively controlled the growth of *P. aeruginosa*. The potential antibacterial activity of *Viola pilosa* shoots extract against *P. aeruginosa* showed that the highest zone of inhibition was measured by ethyl acetate extracted fraction at 2 mg disc\(^{-1}\) concentration. However, no activity was shown by aqueous extracted samples against the same microbe when compared with controls. Roshan et al. (2014), Fazal et al. (2012), Borchardt et al. (2008), Muhammad et al. (2013), Banaszczak et al. (2005) and Gautam et al. (2012) also reported that there is almost no or very less activity showed by aqueous extracted nanoparticles. The results of antibacterial activity of *Skimmia laureola* stem extracts against *P. aeruginosa* exposed that maximum growth inhibition of was noted by ethyl acetate extracted samples at 2 mg disc\(^{-1}\) concentration. The results further revealed that no activity was shown by water extract against *P. aeruginosa*. The potential antibacterial activity of *Skimmia Laureola* leaves extract against *P. aeruginosa* showed that the highest zone of inhibition was measured by ethyl acetate extracted fraction at 2 mg disc\(^{-1}\) concentration. However, no activity was shown by aqueous extracted samples against the same microbe when compared with controls.

**Antibacterial activity of plant extracts against *S. aureus***

The activity of *Viola pilosa* roots extracts against *S. aureus* showed that butanol extracted fraction was effective to control the growth of the tested bacterium at the highest concentration of 2 mg disc\(^{-1}\). The data further suggested that aqueous extracted samples from the tested plant did not reduce the activity of the same microbe at any concentration used measuring 0% ZI. Our results are in agreement
with Arora et al. (2007) who reported antibacterial activity of some Indian medicinal plants against *S. aureus*. The antimicrobial activity of *Viola pilosa* shoots against *S. aureus* indicated that ethyl acetate extracted fraction was more sensitive to inhibit the growth of the tested microbe at the maximum concentration of 2 mg disc\(^{-1}\). Our data also suggested that the butanol and water extracted fractions from the tested shoots did not control the activity of the same bacterium at any concentration used. These results corresponds to those reported by Khan et al. (2011) and Vuuren et al. (2008) who concluded that crude methanolic extracts of various medicinal plants was effective against *S. aureus*. The activity of different extracted samples of *Skimmia Laureola* stem against *S. aureus* revealed that ethyl acetate extracted fraction was effective to control the growth of the tested bacterium at the highest concentration of 2 mg disc\(^{-1}\). The data further suggested that aqueous and butanol extracted samples from the tested plant did not reduce the activity of the same microbe at any concentration used. The results obtained from *Skimmia Laureola* leaves against *S. aureus* indicated that ethyl acetate extracted fraction was more sensitive to inhibit the growth of the tested microbe at the maximum concentration of 2 mg disc\(^{-1}\). The data also suggested that the water extracted fractions from the tested leaves did not control the activity of the same bacterium at any concentration used. Shah et al. (2013) reported similar findings by comparing aqueous extracted fractions and ethyl acetate extracted fractions.

**Antibacterial activity of plant extracts against *B. subtilis***

Data specified for *Viola pilosa* roots samples showed that the highest growth inhibition was measured by butanol at 2 mg disc\(^{-1}\). It is also clear from the result that lowest antimicrobial activity of was revealed by aqueous extracted fraction at 0.5 mg disc\(^{-1}\). These results are in agreement with Sahin et al. (2003) and Bakht et al (2014b), showing similar findings of low or no activity by water extracted fractions. The results obtained from *Viola pilosa* shoot showed that all the extratcs inhibited the growth of *Bacillus subtilis* except aqueous-extracted fraction which did not show activity at any concentration. The highest zone of inhibition of 35.94% was measured by n-hexane at the maximum concentration of 2 mg discs\(^{-1}\). Xie et al. (2004), and Prasad. (2014) showed that n-hexane has higher inhibition against Bacillus subtilis. The analysis *Skimmia Laureola* stem against *B. subtilis* shows that highest growth
inhibition was measured by ethyl acetate at 2 mg disc\(^{-1}\). The data also revealed that the aqueous extract was incapable to show activity against \textit{B. subtilis}. The results from \textit{Skimmia Laureola} leaves present that the highest zone of inhibition against \textit{Bacillus subtilis} was measured by ethyl acetate at the maximum concentration of 2 mg discs\(^{-1}\), while no activity was recorded for aqueous extracted fraction at any concentration.

**Antibacterial activity of plant extracts against \textit{E. coli}**

The results indicated that \textit{E. coli} was also susceptible to different solvent fractions of \textit{Viola pilosa} roots at all concentrations. Maximum growth inhibition of was measured by ethyl acetate fraction at the highest concentration of 2 mg disc\(^{-1}\). Minimum activity of was noted for the same microbe by aqueous extracted samples at the lowest concentration of 0.5 mg disc\(^{-1}\). Muhammad et al., (2012c) showed resistance of \textit{E. coli} against the water extracted fractions. The data showed that \textit{Escherichia coli} showed maximum susceptibility to n-hexane extracted fractions of \textit{Viola pilosa} shoots at the higher concentration of 2 mg disc\(^{-1}\). The results further revealed that no activity was shown by butanol and water extracted fraction against \textit{E. coli}. Muluye et al., (2014) and Pranting et al., (2010) showed very low activity of butanol and water extracts against microbes. The results also indicated that \textit{E. coli} was also susceptible to different solvent fractions of \textit{Skimmia Laureola} stem. Maximum growth inhibition was measured by ethyl acetate fraction at the highest concentration of 2 mg disc\(^{-1}\). No activity was noted for the same microbe by aqueous and butanol extracted samples at all the given concentrations. \textit{Escherichia coli} showed maximum susceptibility to ethyl acetate extracted fractions of \textit{Skimmia Laureola} leaves measuring highest growth inhibition at the higher concentration of 2 mg disc\(^{-1}\). The results further revealed that no activity was shown by butanol and water extracted fraction against \textit{E. coli}. Mehmood et al. in 2013, showed higher growth inhibition by ethyl acetate extration.

**Antibacterial activity of plant extracts against \textit{Xanthomonas campestris}**

The antibacterial activity of different solvent extracted samples from the roots of \textit{Viola pilosa} revealed that the growth of \textit{X. campestris} was inhibited by different
solvent extracted fraction at all concentrations. Highest activity was exhibited by butanol extracted fraction at 2 mg disc\(^{-1}\). The results further indicated that aqueous extracted samples were less effective to control the activity of the same microbe at 0.5 mg disc\(^{-1}\) measuring 27.58% ZI. In 2003, Karaman et al, found the same results of highest activity of butanol-extracted fractions of roots. The antibacterial activity of shoots of *Viola pilosa* showed that *Xanthomonas campestris* was found resistant against aqueous extracts showing no activity at any concentration. Maximum inhibition was showed by n-butanol extracts at 2 mg disc\(^{-1}\). Mahesh and Satish in 2008 and Roshan et al. in 2014 showed that antimicrobial activity is minimal against butanol and maximum against water extracted fractions. The data from stem of *Skimmia* revealed that the highest activity was exhibited by ethanol-extracted fraction at 2 mg disc\(^{-1}\). The results further indicated that aqueous extracted samples were not effective to control the activity of the same microbe at any concentration. The antibacterial activity of different solvent extracted samples from the leaves of *Skimmia Laureola* against *Xanthomonas campestris* was found resistant against aqueous extracts showing no activity at any concentration. Maximum inhibition was showed by ethyl acetate extracts at 2 mg disc\(^{-1}\). Fazal et al. (2012) verified that this microbe was fully susceptible to ethyl acetate fractions.

**Antibacterial activity of plant extracts against *K. pneumonia***

*Klebsiella pneumoniae*, (Gram negative bacteria), was inspected for its efficiency against different extracts. This microbe is accountable for diseases like bronchitis and pneumonia. Its causes severe changes in the lungs through inflammation and bleeding. Sometimes it causes internal damages to lungs that result in severe breathing problems. The antibacterial activity of different solvent extracted samples from the roots of *Viola pilosa* against *K. pneumoniae* indicated that the tested microbe was completely resistant to all the solvent tested at all the concentrations measuring 0% ZI. Adhikary et al. (2011) concluded that crude methanolic extracted samples of *Viola pilosa* did not show antibacterial activity against *Klebsiella sp*. The antibacterial activity of different solvent extracted samples from the shoots of *Viola pilosa* against *K. pneumoniae* presented high zone of maximum inhibitory zone by butanol extracted fraction at a concentration of 2 mg disc\(^{-1}\). However aqueous extracts presented no activity when compared with controls and other extracts. Daoud et al.
(2012) reported the highest zone of inhibition of butanol extracted against *K. pneumonia*. The results from stem of *Skimmia Laureola* against *K. pneumoniae* indicated that the aqueous extract was incapable to show any activity at all the tested concentrations. Maximum growth of inhibition was noticed for ethyl acetate at a concentration of 2 mg disc$^{-1}$. The antibacterial activity of different solvent extracted samples from the leaves of *Skimmia Laureola* against *K. pneumoniae* stated that the maximum inhibitory zone of was measured by ethyl acetate extracted fraction at a concentration of 2 mg disc$^{-1}$ and the lowermost by butanol extracted samples at a concentration of 0.5 mg disc$^{-1}$. However aqueous extracts presented no activity when compared with controls and other extracts Savithramma et al. (2011) and Tona et al. (1998).

**Antifungal activity and anti-yeast potential of gold and silver nanoparticles (AuNPs and AgNPs) and various extracts of Viola pilosa and Skimmia laureola.**

The current study was also designed to investigate the antifungal and anti-yeast potential of different solvent extracted samples and their respective gold and silver nanoparticles (AuNPs and AgNPs) from the roots and shoots of *Viola pilosa* and stem and leaves of *Skimmia laureola*. Six different fungal strains were tested against all these extracts. Three different concentrations of 0.5mg, 1mg and 2mg were selected for testing the microbes against plants extracts and their AuNPs and AgNPs. The antifungal activities were determined through well diffusion method.

The statistical analysis of the data concerning the antifungal and anti-yeast potential of silver nanoparticles of crude methanolic extract AgNPs of *Viola pilosa* roots by well diffusion method stated that the maximum level of sensitivity to the nanoparticles was discovered in *A.niger* at 18µl disc$^{-1}$. However lowest inhibition value was noticed at 6µl disc$^{-1}$ concentration against *C. albicans*. The statistical data concluded that that the antifungal and antiyeast activities were purely dependent on dose. The increase in activity was noticed with an increase in the concentration level of silver nanoparticles. The results obtained from the aqueous roots extract AgNPs of *Viola pilosa* discovered that extreme zone of inhibition was presented by *Rhizopus* at 18µl disc$^{-1}$. The lowest inhibitory zone was noticed for *C. albicans* at a concentration of 6µl disc$^{-1}$. The results of silver nanoparticles of crude methanolic extract of *Viola*
Viola pilosa shoots stated that the maximum level of sensitivity of was discovered in *Rhizopus* at 18µl disc⁻¹. However lowest inhibition value was noticed at 6µl disc⁻¹ concentration against *C. albicans*. The results obtained from the AgNPs made from the aqueous shoots extract of *Viola pilosa* discovered that extreme zone of inhibition was presented by *Curvularia* at 18µl disc⁻¹. The lowest inhibitory zone of 26.66% was noticed for *C. albicans* at a concentration of 6µl disc⁻¹ (Jonny et al., 2014).

The statistical data obtained from the crude extract of *Skimmia laureola* stems showed that the maximum reduction in growth of was noticed against *Paecilomyces* and the lowermost value of growth inhibition was observed for *C. albicans* at 6mg disc⁻¹ concentration. The results of nanoparticles made from the aqueous extract of *Skimmia laureola* stems revealed that the maximum reduction in growth of was noticed against *A. niger* and *Alterneria*. The lowermost value of growth inhibition was observed for *C. albicans* at 6mg disc⁻¹ concentration. The statistical data of nanoparticles from the crude extract of *Skimmia laureola* leaves exposed that the maximum reduction in growth was noticed against *A.niger* and *Alterneria*. The lowermost value of growth inhibition was observed for *C. albicans* at 6mg disc⁻¹ concentration. The nanoparticles made from the aqueous extract of *Skimmia laureola* leaves showed that the maximum reduction in growth was noticed against *A. niger* while the lowermost value of growth inhibition was observed for *C. albicans* at 6mg disc⁻¹ concentration. Barkatullah et al. (2015) reported similar findings of maximum reduction against *A. niger* and lowest against *C. albicans* at 6mg disc⁻¹ concentration.

The statistical results of the data about the antifungal and anti-yeast potential of gold nanoparticles of crude methanolic extracts of *Viola pilosa* roots extract AuNPs specified that the extreme level of sensitivity of was discovered in *Paecilomyces* at 18µl disc⁻¹. However lowest inhibition value was noticed at 6µl disc⁻¹ concentration against *C. albicans*. The results obtained from AuNPs made from the roots extract of *Viola pilosa* against five fungal strains and one yeast strain discovered that highest zone of inhibition was presented by *Paecilomyces* at 18µl disc⁻¹. The lowest inhibitory zone of was noticed for *C. albicans* at a concentration of 6µl disc⁻¹. The statistical results of the data of gold nanoparticles of crude methanolic extracts of *Viola pilosa* shoots specified that the extreme level of sensitivity was discovered in *Paecilomyces* at 18µl disc⁻¹. However lowest inhibition value was noticed at 6µl disc⁻¹.
concentration against *C. albicans*. The results of antifungal and anti-yeast properties of AuNPs from the roots extract of *Viola pilosa* discovered that highest zone of inhibition was presented by *Rhizopus* at 18µl disc<sup>-1</sup>. The lowest inhibitory zone of was noticed for *C. albicans* at a concentration of 6µl disc<sup>-1</sup>. Jayaseelan et al. (2013) conducted study on antifungal activity of AuNPs and stated that all the strains were susceptible.

The statistical data of the antifungal and anti-yeast activity of gold nanoparticles obtained from the crude extract of *Skimmia laureola* stems presented that the maximum reduction in growth was noticed against *Alternaria*. The lowermost value of growth inhibition 30% was observed for *C. albicans* at 6mg disc<sup>-1</sup> concentration. The antifungal activity of nanoparticles made from the aqueous extract of *Skimmia laureola* stem illustrated that the maximum reduction in growth was noticed against *C. albicans*. The lowermost value of growth inhibition was observed for *A.niger* at 6mg disc<sup>-1</sup> concentration. The data of nanoparticles from crude extract of *Skimmia laureola* leaves showed that the maximum reduction in growth was noticed against *Paecilomyces*. The lowermost value of growth inhibition was observed for *C. albicans* at 6mg disc<sup>-1</sup> concentration. The results from the aqueous extract of *Skimmia laureola* stem exposed that maximum reduction in growth of was noticed against *Paecilomyces* at 18µl disc<sup>-1</sup>. The lowermost value of growth inhibition 26.66% was observed for *C.albicans* at 6mg disc<sup>-1</sup> concentration. The statistical results recommended that the antifungal and antiyeast properties were dependent on dose and increase in activity was noticed upon increase in concentration of the nanoparticles. Jayaseelan et al. (2013) found that all the fungal strains were susceptible to the AuNPs.

**Antifungal potential of plant extracts against Candida albicans**

The results of the antifungal activity of *Viola pilosa* root extracts against *C. albicans* exposed that maximum growth inhibition was noted by hexane extracted samples at 2 mg disc<sup>-1</sup> concentration. Similarly, minimum activity against the same microbe was measured by aqueous extracted samples at 0.5 mg disc<sup>-1</sup> compared with other samples and controls. The antifungal activity of different solvent extracted samples from the shoots of *Viola pilosa* against *C.albicans* stated that the maximum
inhibitory zone was measured by ethyl acetate and n-hexane extracted fraction at a concentration of 2 mg disc\(^{-1}\) and the lowermost by aqueous extracted samples at a concentration of 0.5 mg disc\(^{-1}\). The results of antifungal activity of *Skimmia laureola* leaves extracts against *C. albicans* exposed that the maximum growth inhibition was noted by hexane extracted samples at 2 mg disc\(^{-1}\) concentration. The results further revealed that no activity was shown by water extract against *C. albicans*. The potential antifungal activity of *Skimmia Laureola* stem extract against *C. albicans* showed that the highest zone of inhibition of was measured by hexane extracted fraction at 2 mg disc\(^{-1}\) concentration. However, low activity was shown by butanol extracted samples at 0.5 mg disc\(^{-1}\) concentration against the same microbe when compared with controls. Rana and Jain. (2011) showed that the maximum inhibition zone measured by hexane extracted fraction and low by butanol extracted samples.

**Antifungal potential of plant extracts against *Alternaria solani***

Data obtained from *Viola pilosa* root extracts indicated that the highest growth inhibition was measured by crude at 2 mg disc\(^{-1}\) and the lowest antifungal activity was revealed by aqueous extracted fraction at 0.5 mg disc\(^{-1}\). The potential antifungal activity of *Viola pilosa* shoots extracts against *Alterneria* showed that the highest zone of inhibition was measured by aqueous extracted fraction at 2 mg disc\(^{-1}\) concentration. However, minimum activity was shown by hexane extracted samples at 0.5 mg disc\(^{-1}\) concentration. The activity of *Skimmia Laureola* leaves against *Alterneria* revealed that aqueous and butanol extracted fractions were effective to control the growth of the tested fungus at the highest concentration of 2 mg disc\(^{-1}\). The results from the stem of *Skimmia Laureola* against *Alterneria* reported that the maximum inhibition was showed by butanol and aqueous extracts at 2 mg disc\(^{-1}\). Aslam et al. (2010) also worked with *Alterneria* and found it susceptible to the plant extracts.

**Antifungal potential of plant extracts against *Paecilomyces***

The activity of different extracted samples of *Viola pilosa* roots against *Phacelomyces* revealed that hexane extracted fraction was effective to control the growth of the tested fungus at the highest concentration of 2 mg disc\(^{-1}\). The potential
antifungal activity of *Viola pilosa* shoots showed highest growth inhibition activity by hexane at the higher concentration of 2 mg disc\(^{-1}\). The results of *Skimmia Laureola* stems indicated that the maximum growth inhibition was measured by ethyl acetate fraction at the highest concentration of 2 mg disc\(^{-1}\). The lowest activity were noted for the same microbe by aqueous and crude extracted samples. The activity of *Skimmia Laureola* leaves illustrated that the highest zone of inhibition against *Paecilomyces* was measured by hexane at the maximum concentration of 2 mg discs\(^{-1}\).

**Antifungal potential of plant extracts against *Curvularia***

The data from antifungal activity of *Viola pilosa* roots revealed that highest activity was exhibited by aqueous extracted fraction at 2 mg disc\(^{-1}\). The data obtained from *Viola pilosa* shoots illustrated that the highest zone of inhibition was measured by n-hexane at the maximum concentration of 2 mg discs\(^{-1}\). The lowest zone of inhibition was noted for ethyl acetate at a concentration of 0.5 mg discs\(^{-1}\). The results of *Skimmia Laureola* leaves indicated that highest growth inhibition zone was measured by butanol at 2 mg disc\(^{-1}\). The lowest antifungal activity of was revealed by crude extracted fraction at 0.5 mg disc\(^{-1}\). The antifungal activity of various solvent extracted fractions of *Skimmia Laureola* stem against *Curvularia* showed that the maximum growth of inhibition was noticed for aqueous at a concentration of 2 mg disc\(^{-1}\) and the lowest for ethyl acetate at lowest concentration of 0.5mg disc\(^{-1}\). Our results are in accordance with Sailaja. (2010) who reported significant antifungal activity with *Curvularia* against some wild plants species.

**Antifungal potential of plant extracts against *Rhizopus***

The results of antifungal activity of *Viola pilosa* root extracts against *Rhizopus* exposed that maximum growth inhibition was noted by hexane extracted samples at 2 mg disc\(^{-1}\) concentration. Similarly, minimum activity against the same microbe was measured by aqueous extracted samples at 0.5 mg disc\(^{-1}\). The antifungal activity of different solvent extracted samples from the shoots of *Viola pilosa* revealed that the maximum inhibition was showed by aqueous extracts at 2 mg disc\(^{-1}\). The antifungal activity of different solvent extracted samples from the leaves of *Skimmia Laureola* against *Rhizopus* presented high zone by aqueous extracted fraction at a concentration
of 2 mg disc$^{-1}$ and the lowermost by crude extracted samples 68.02% at a concentration of 0.5 mg disc$^{-1}$. The antifungal activity of samples from the stem of Skimmia Laureola showed that the highest growth inhibition activity was possessed by butanol at the higher concentration of 2 mg disc$^{-1}$. Moghim et al. (2015) worked with Rhizopus and found similar findings of highly susceptible to the antifungal activity.

**Antifungal potential of plant extracts against Niger**

The results from antifungal activity of Viola pilosa root indicated maximum growth inhibition by ethyl acetate at the highest concentration of 2 mg disc$^{-1}$. Minimum activity was noted for the aqueous extracted samples at the lowest concentration of 1 mg disc$^{-1}$. The activity of Viola pilosa shoot extracts showed that butanol extracted fraction was more sensitive to inhibit the growth at the maximum concentration of 2 mg disc$^{-1}$. The antifungal activity of different solvent extracted samples from the leaves of Skimmia Laureola demonstrated maximum growth of inhibition by aqueous extracted fraction at a concentration of 2 mg disc$^{-1}$. Data obtained from stem of Skimmia Laureola revealed highest growth inhibition by aqueous fraction at 2 mg disc$^{-1}$. It is also clear from the result that lowest antifungal activity was revealed by ethyl acetate extracted fraction at 0.5 mg disc$^{-1}$. Rathod et al. (2015) and Ahmad and sultana. (2003) also reported highest inhibition on high concentration of water extraction, but as compare to ethyl acetate the activity was revealed to be low.

**DPPH Radical Scavenging Activity of gold and silver nanoparticles (AuNPs and AgNPs) and various extracts of Viola pilosa and Skimmia laureola.**

Compounds possessing the ability of scavenging free radicals are formed naturally by the plants. These compounds are lignins, phenolic acids, flavonoids, tannins, terpenoids, stilbenes, alkaloids coumarins, and vitamins etc. (Cait et al., 2003). Consumption of naturally produced antioxidants by diet or through herbal medicine can decrease the risk of cancer, cardiac disease and other problems associated with free radicals (Veerapur et al., 2009).
The antioxidant potential of different extracts from the roots and shoots of *Viola* as well as from the leaves and stem of *Skimmia* was evaluated in DPPH radical scavenging bioassay. Each extract was used in four different concentrations (25, 50, 125 and 250 µg/ml) and their radical scavenging activity was assessed against the 98.95% activity exhibited by the standard, Gallic acid (500 µg/ml). The data showed all the fractions extracted with different solvents and their nanoparticles displayed antioxidant activity at both the lower and higher concentrations when compared to the positive control.

All tested concentrations of each *Viola* root extract were active in scavenging free radicals when evaluated in DPPH radical scavenging assay. Ethyl acetate fraction with activities was by far the most potent of the tested extracts at 250 µg/ml. Butanol (250 µg/ml), aqueous (250 µg/ml) and methanol (250 µg/ml) followed suit in a sequential manner. (Hajji et al., 2009) and (Kumar et al., 2016). Hexane fraction, on the other hand, measured the least activities in comparison to the other tested extracts at 25µg/ml. The results of *Viola* shoot extracts revealed that the least potent among the tested extracts turned out to be hexane fraction at 25 µg/ml while the best antioxidant activity was revealed by the sample extracted with butanol at 250 µg/ml. (Jamshed et al., 2012). All samples extracted from the leaves of *Skimmia* were active in scavenging free radicals at each of the four concentrations used. The best radical scavenging activity was depicted by butanol fraction at 250µg/ml. The lowest activities noted were at 25 µg/ml which were exhibited by the sample extracted with hexane. Five samples in different solvents were also extracted from the stem of *Skimmia* and all showed antioxidant activity at each tested concentration. Ethyl acetate fraction revealed maximum scavenging activity. Crude methanol extract turned out to be the least potent among the tested extracts in scavenging free radicals at 25 µg/ml. The outcomes of whole data also suggested that all the plant extracts presented significant antioxidant potential even at lowest concentration of 25µg/ml (Jamshed et al., 2012).

The data obtained from silver nanoparticles of aqueous and crude extracts of *Viola* revealed the presence of antioxidant activity for all tested silver nanoparticles at each concentration used. The least potent among the tested nanoparticles turned out to be *Viola* root aqueous extract at 25 µg/ml while the best antioxidant activity was
revealed by the nanoparticles extracted with *Viola* shoots aqueous fraction measuring activities of at 250µg/ml. The results of silver nanoparticles extracted from the leaves and stem of *Skimmia* verified that the best radical scavenging activity was depicted by *skimmia* stem aqueous 250 µg/ml. The lowest activities noted were at 25 µg/ml respectively which were exhibited by the nanoparticles extracted with *Skimmia* leaves crude fraction. Chung et al. (2016) reported higher anti-microbial activity in crude extracts of plant. The antioxidant activity of gold nanoparticle of *Viola pilosa* revealed that the *Viola* roots aqueous extracted nanoparticles at 250 µg/ml were by far the most potent of the tested extracts in scavenging free radicals. *Viola* shoot crude fraction, on the other hand, measured the least activities in comparison to the other tested nanoparticles at 25 µg/ml.

Gold nanoparticles from the stem and leaves of *Skimmia* revealed higher antioxidant activity at 250 µg/ml. *Skimmia* stem crude extract measured lowest activity at 25 µg/ml and, hence, turned out to be the least potent among the tested nanoparticles extracts in scavenging free radicals.

**Phytotoxic activity of plant extracts**

Weeds infestation is a major problem of crops faced by the famers for which need help from chemical herbicides that are naturally offensive, exclusively to soil, water and food (Wahab et al., 2012). The current study was designed to carry out phytotoxic evaluation for development of herbicides that are cost friendly and environmentally safe. The data attained from the phytotoxic bioassay of solvent extracts from the root of *Viola pilosa* revealed a significant phytotoxic level for all the solvent extracts samples against *Lemna minor*. The statistical data specified that amongst these fractions, the highest level of phytotoxicity at 18µl disc⁻¹ was showed by crude methanolic extract. The lowest phytotoxic potential of was noticed for the aqueous extracted fraction at a concentration of 6µl disc⁻¹. The sequence of the phytotoxic level of the five solvent extracted fractions was methanolic crude > ethyl acetate > n- butanol and n-hexane > aqueous. The data obtained from the shoots of *Viola pilosa* plant also exposed a significant phytotoxic level for all the solvent extracts samples against *Lemna minor*. The highest level of phytotoxicity at 18µl disc⁻¹ was showed by hexane extract. The lowest phytotoxic potential was noticed for the
aqueous and crude methanolic extracted fractions at a concentration of 6µl disc⁻¹. The sequence of the phytotoxic level of the five solvent extracted fractions was hexane > butanol > ethyl acetate > crude > aqueous. Grisi et al. (2013) reported significant phytotoxic potential of hexane extracted samples.

The data attained from stem of *Skimmia laueola* showed that the highest level of phytotoxicity at 18µl disc⁻¹ was showed by aqueous extract. The lowest phytotoxic potential was noticed for the hexane extracted fraction at a concentration of 6µl disc⁻¹. The sequence of the phytotoxic level of the five solvent extracted fractions was methanolic aqueous > butanol > crude > ethyl acetate > n-hexane. The results of the phytotoxic bioassay of solvent extracts from the leaves of *Skimmia laureola* plant exposed the highest level of phytotoxicity at 18µl disc⁻¹ showed by aqueous extract. The lowest phytotoxic potential was noticed for the crude methanolic fraction at a concentration of 6µl disc⁻¹. The sequence of the phytotoxic level of the five solvent extracted fractions was aqueous > ethyl acetate > hexane > butanol > crude. Rahmaullah et al. (2012) and Rauf et al. (2012) determined higher level of phytotoxicity in crude extracts and lowest in aqueous extracts.

**Insecticidal activity of plant extracts**

The stored grains insects are a serious threat to the crops. Even if the crop is not entirely damaged, the nutritional value of grain is badly affected by pest infestation. The grain embryo is targeted by these insects that results in reducing protein content percentage of seeds germination (Department of Agriculture and Food, Government of Australia). Excessive use of chemical insecticides should be discouraged because of the unfavorable environmental effects. There is a dire need of developing novel, cost effective and ecofriendly insecticides to overcome this problem. The results regarding the insecticidal potential achieved from the five solvents extracts from the roots of *Viola pilosa* plant against *Tribolium castaneum* showed that the maximum mortality rate was shown by ethyl acetate at a concentration of 18µl disc⁻¹ while hexane and butanol fractions did not show any mortality at 6µl disc⁻¹. The data from the extracts of the shoots of *Viola pilosa* plant against *Tribolium castaneum* revealed that the maximum mortality rate was shown by crude at a concentration of 18µl disc⁻¹ while butanol and aqueous fractions did not
show any mortality at 6µl disc\(^{-1}\). The results from the stem of *Skimmia laureola* plant against *Tribolium castaneum* show the maximum mortality rate by ethyl acetate at a concentration of 18µl disc\(^{-1}\). The butanol and crude fractions did not show any mortality at 6µl disc\(^{-1}\) and 12µl disc\(^{-1}\). Data from the leaves of *Skimmia laureola* plant against *Tribolium castaneum* shows the maximum mortality rate by hexane and aqueous at a concentration of 18µl disc\(^{-1}\). The ethyl acetate, butanol and crude fractions did not show any mortality at 6µl disc\(^{-1}\) and 12µl disc\(^{-1}\). The study revealed that all the five solvent extracted fractions presented variable and significant insect and dose dependent insecticidal capability (Chen et al., 2007; Chu et al., 2012).

The results achieved from the insecticidal potential from the five solvents extracts from the roots of *Viola pilosa* plant against *S. oryzae* showed the maximum mortality rate by crude and ethyl acetate at a concentration of 18µl disc\(^{-1}\), while no activity was shown by ethyl acetate, aqueous and butanol fractions at 6µl disc\(^{-1}\). The results achieved from shoots of *Viola pilosa* plant against *S. oryzae* showed the maximum mortality rate by hexane at a concentration of 18µl disc\(^{-1}\), whereas crude, ethyl acetate and aqueous fractions did not show any mortality at 6µl disc\(^{-1}\). The primary analysis of stem of *Skimmia laureola* revealed that the maximum mortality rate was shown by butanol and hexane at a concentration of 18µl disc\(^{-1}\). Whereas crude, hexane and ethyl acetate fractions did not show any mortality at 6µl disc\(^{-1}\). The results achieved from the insecticidal potential from the leaves of *Skimmia laureola* against *S. oryzae* presented the maximum mortality rate at a concentration of 18µl disc\(^{-1}\). The data also suggested that no mortality was shown by crude, aqueous, ethyl acetate and hexane fractions at 6µl disc\(^{-1}\). Saleem et al. (2004), Jangwan et al. (2010), Wang et al. (2010) and Rehmanullah et al. (2012) all reported the best insecticidal activity of crud extracts of plant. Based on these results, the polar solvents like water and butanol can be used for isolating insecticidal mixtures for developing commercial insecticides (Chu et al., 2012).

**Phyto chemistry of plant extracts**

The utilization of plants species for treatment of wide spread of diseases is attributed to the presence of numerous bioactive compounds (Ayodele, 2003). Bioactive compounds are used by the plants in the form of secondary metabolites for safety and repair processes. Most important antioxidants produced by plants include Phenols, flavonoids and tannins which possess anti-diarrheal activities (Agbor et al.,
2004) and are also helpful in controlling conditions associated to oxidative trauma (Vinson et al., 1995). Tannins are recognized by tremendous antibacterial activities and generate immune response against many parasites (Tiger, 1980). Flavonoids are well known for anticancerous properties and also have good antioxidant activity (Okwu, 2006). The alkaloids in various extracts can be related to the antimicrobial potentials (Ramkumar et al., 2007). These bioactive compounds can be extracted from any part of plants like seeds, flowers, stem, leaves, bark and roots etc. Any portion might contain active components (secondary metabolites) in low or high amounts (Ara and Nur, 2009).

In the current study different solvent extracted samples from Viola pilosa and Skimmia laureola were subjected to phytochemical screening for the presence of Alkaloids, flavonoids, proteins, fats, oils, tannins, carbohydrates, sterols and saponins according to the standard procedures of analysis. The results achieved from phytochemical study of the shoot of Viola pilosa discovered that proteins, tannins, sterols, flavonoids and carbohydrates were found in all the extracts. Alkaloids were absent in hexane and ethyl acetate fractions, saponins similar fats and oils were absent in ethyl acetate fraction. In case of Viola pilosa saponins, sterols, flavonoids and carbohydrates were found in all the extracts. Alkaloids were absent in hexane and ethyl acetate fractions, proteins and tannins in hexane and fats and oils were absent in ethyl acetate fraction. Kumar et al. (2011) and Adhikary et al. (2011) showed proteins, sterols, flavonoids and carbohydrates in all solvent extracted samples of plant.

The results obtained from the stem of Skimmia laureola revealed the presence of sterols, flavonoids and tannins in all the fractions whereas saponins were absent in ethyl acetate extracted samples. However, lipids were found abundant in crude and hexane extracted fractions and were found absent in n-butanol, aqueous and ethyl acetate fractions. The results also demonstrated the moderate presence of alkaloids in crude and butanol extracts while showing negative results for n-hexane, ethyl acetate and aqueous extracts respectively. The results from the leaves of Skimmia laureola discovered the presence of carbohydrates, proteins and tannins in all the fractions whereas absence of alkaloids, lipids and flavonoids in aqueous extracted samples. Sterols were found absent in n-hexane and ethyl acetate fractions. The phytochemical analysis of the leaves extracts also demonstrated absence of saponins in butanol and ethyl acetate extracts respectively.
VI. SUMMARY

*Viola pilosa* or *Viola serpens* is a member of family Violaceae (commonly known as Leoniaceae, Alsodeiace or Retrosepalaceae) which include twenty genres and around 800 species. *Viola* belongs to the genus of flowering plants in the violet family Violaceae. It is the major genus in the violet family covering about 525 to 600 species. In Pakistan, it is characterized by genus (*Viola*) and 17 different species and is usually found in Siran and Swat valleys. *Viola pilosa* or *Viola serpens* Wall, is generally known as smooth leaf white violet or “Banafsha”. *Viola pilosa* has been used to treat innumerable infections. It is useful in asthma, bleeding piles, cancer of throat, constipation, cough, fever, headache, and skin diseases. The whole plant of *Viola serpens* wall is taken as decoction orally as a remedy for hepatitis and jaundice.

*Skimmia laureola* (*S. laureola*) typically named as Nazar Panra is a member of family Rutaceae. In Pakistan, it is commonly found in region of Hazara, Murree, Kashmir valley, Shangla and upper Swat. Healing properties of the *S. laureola* plant have been acknowledged in different cultures of the world. It has been used in the treatment of headache, cold and fever. Dry leaves smoke has been used as a treatment for nasal tract blockage. Moreover, leaves of *Skimmia laureola* have been commended for several other purposes like cough relieve and as insecticide and pesticide. Leaves from the plant are harvested and commercially used in different food stuffs as additives, in old-fashioned healing and traditional practices, being made in garlands and considered as sacred. A complex anthelmintic property was observed in the essential oils extracted from the plant.

The gold and silver nanoparticles (AuNPs and AgNPs) were successfully synthesized from solvent extracted fractions of the *Viola pilosa* and *Skimmia laureola*. During the course of study it was confirmed that both plants have probable antifungal and antibacterial potentials. An excellent amount of antioxidant activity was also demonstrated by the extracts of plants. The plant extracts of both plants were also assessed qualitatively that confirmed the existence of significant bioactive compounds which are powerful antifungal, antibacterial and antioxidant agents.
In the current study, a solution of .1 mM of AuCl₃ was used for the bioinspired production of gold nanoparticles. The obtained spectrum specified that the highest peak was observed by the combination of 4ml of AuCl₃ solution and 1ml of the plant crude root extract (4:1). The color of the pure plant extracts (Aqueous, crude methanol and ethanol) changed to dark purple from yellowish with the addition of .1mM AuCl₃ which confirmed the formation of AuNPs, which was reconfirmed by spectrophotometer. The gold nanoparticles of Viola were found highly stable at the temperature range between 25°C and 50°C and that of Skimmia were stable at the temperature range between 20°C and 40°C. An increase in temperature reduced the stability of the gold nanoparticles. The AuNPs were found more stable at salt stress in milli-Molar concentrations as compared to molar concentrations. The characterization of gold nanoparticles was carried out by using FT-IR, XRD and SEM. The FT-IR study verified that phenols and esters were accountable for the green synthesis of the gold nanoparticles (AuNPs). The X-Ray diffraction confirmation of the powdered gold nanoparticles showed crystalline structure, and centro symmetric nature. The results of SEM on AuNPs confirmed the size of smallest particle to be 20 nm and that of largest particle to be 50nm.

In the present research, silver nanoparticles (AgNPs) were also synthesized. A solution of .1 mM of AgNO₃ was used for the bioinspired production of silver nanoparticles. The obtained spectrum specified that the highest peak was observed by the combination of 1ml of AgNO₃ solution and 10ml of the plant crude root extract (1:10). The color of the pure plant extracts (Aqueous, crude methanol and ethanol) changed to dark brown from yellow with the addition of .1mM AgNO₃ which confirmed the formation of AgNPs, which was reconfirmed by spectrophotometer. The silver nanoparticles of Viola were found highly stable at the temperature range between 25°C and 50°C and that of Skimmia were stable at the temperature range between 20°C and 40°C. An increase in temperature reduced the stability of the silver nanoparticles. The AgNPs were found more stable at salt stress in milli-Molar concentrations as compared to molar concentrations. The characterization of silver nanoparticles was carried out by using FT-IR, XRD and SEM. The FT-IR study verified that phenols and esters were accountable for the green synthesis of the silver nanoparticles (AgNPs). The X-Ray diffraction confirmation of the powdered silver
nanoparticles showed crystalline structure, and centro symmetric nature. The results of SEM on AgNPs confirmed the size of smallest particle to be 20 nm and that of largest particle to be 80nm.

The current study was also designed to investigate the antibacterial activities of different solvent extracted samples and their respective gold and silver nanoparticles (AuNPs and AgNPs) from the roots and shoots of Viola pilosa and stem and leaves of Skimmia laureola. It was revealed that the efficiency of the plant extracts against microbial strains was increased by the nanoparticles. The silver nanoparticles (AgNPs) were found more effective in controlling the growth of bacterial and fungal strains as compared to gold nanoparticles (AuNPs). Significant and moderate (in some cases) amount of activities were recorded by both types of nanoparticles against all the tested bacterial and fungal strains at concentrations of 0.5 mg, 1mg and 2 mg.

The data attained from Viola pilosa root extracts against P. aeruginosa, E. coli, B. subtilis and Xanthomonas compestris exposed that different solvent extracted samples were completely active against these strains and measured varying degree of growth inhibition at all the tested concentrations. Data obtained from S. aureus showed that it was not found sensitive to aqueous extract. On the other hand, the results against K. pneumonia indicated that the tested microbe was completely resistant to all the solvent extracts tested at all the concentrations. However, the results achieved from the shoot extracts of Viola pilosa against P.aeruginosa, B. subtilis, K. pneumonia and Xanthomonas compestris showed that the microbes were susceptible to the solvent extracted fractions except the aqueous extract that did not show any activity against the microbe at all the three concentrations under study. The results of data obtained from E. coli and S. aureus showed that they were found totally resistant against the n-butanol and aqueous extracted fractions.

The results of antibacterial activity of Skimmia laureola stem extracts against P. aeruginosa, B. subtilis, Xanthomonas compestris and K. pneumonia demonstrated that solvent extracted samples presented different degree of growth inhibition at all the three tested concentrations. These microbes were found susceptible to all the solvent extracted fractions except the aqueous extract that did not show any activity
against these microbes. The results further showed that *E. coli* and *S. aureus* were found totally resistant against the n-butanol and aqueous extracted fractions. The potential antibacterial activity of *Skimmia Laureola* leaves extract against *P. aeruginosa, B. subtilis, K. pneumonia, Xanthomonas compestris* and *S. aureus* revealed that these strains were susceptible to all the solvent extracted fractions except the aqueous that was unable to show any activity at the concentrations under study. However, *E. coli* was declared totally resistant against the aqueous and n-butanol extracted fractions.

Solvent extracted fractions from *Viola pilosa* root and shoots extracts were found to be vigorously active against the six fungal strains at all the three concentrations. Variable amount of activity was shown by different extracts of *Viola* against the three concentrations. The antifungal activity of different extracted samples of *Skimmia Laureola* stem and leaves against *Alternaria, Curvularia, A. niger, Paecilomyces* and *Rhizopus* discovered that the tested strains were completely susceptible to all the solvent extracted samples and were capable to show significant activity at all the three concentrations. However, stem and leaves aqueous extracts were found inactive against *C. albicans* and were not found effective to control the growth of the tested strain.

The antioxidant potential of gold and silver nanoparticles (AuNPs, AgNPs) from the roots and shoots of *Viola* as well as from the leaves and stem of *Skimmia* was evaluated in DPPH radical scavenging bioassay. All tested concentrations of silver and gold nanoparticle from both plants were active in scavenging free radicals possessing significant antioxidant potential when compared to the control. Similarly, roots extracts of *Viola pilosa* were by far the most potent in scavenging free radicals amongst all the other solvent extracts and showed much significant anioxidant activities. The order of scavenging potential of roots extracts of *Viola pilosa* was ethyl acetate > butanol > aqueous > crude > n-hexane. The data further showed that stem extracts of *Skimmia* revealed better radical scavenging activity as compared to the leaves extracts. The order of radical scavenging potentials of stem extracts of *Skimmia* was ethyl acetate > butanol > aqueous > hexane > crude at all the four concentrations.
The phytotoxic potential of five solvents extracted fractions from of *Viola pilosa* and *Skimmia laureola* was also carried out by the help of *Lemna minor* plant for the verification of the phytotoxic activity of each extract. The analysis of phytotoxicity on both plants revealed a significant phytotoxic level for all the solvent extracts samples against the tested plant. The statistical data specified that amongst these fractions, the highest level of phytotoxicity was showed by stem extracts of *Skimmia laureola* at the highest concentration under study. The primary insecticidal analysis of *Viola pilosa* and *Skimmia laureola* against the test insects revealed that all the five solvent extracted fractions presented variable and significant insect and dose dependent insecticidal capabilities. However, the sequence of activity was different for different parts of both test plants. Some parts showed higher and some showed low activities against variable extracts. Even, in some cases no activity was showed by different plant extracts at the lowest concentration under test. The results achieved from phytochemical study of the test plants discovered the existence of secondary metabolites in crude and other extracts under study, such as alkaloids, flavonoids, proteins, lipids, tannins, carbohydrates, sterols and saponins. The samples were abundant in tannins, carbohydrates, sterols, proteins and lipids.
VII. CONCLUSIONS AND RECOMMENDATIONS

The gold and silver nanoparticles (AuNPs and AgNPs) were successfully synthesized from solvent extracted crude and aqueous fractions of the *Viola pilosa* and *Skimmia laureola* by using biogenic method of nanoparticles synthesis. The current study revealed that the gold and silver nanoparticles of *Viola* were found highly stable at the temperature range between 25°C and 50°C and that of *Skimmia* between 20°C and 40°C and more stable at salt stress in milli-Molar concentrations as compared to molar ones. The X-Ray diffraction confirmation of the powdered gold nanoparticles showed crystalline structure, and centro symmetric nature. Phenols and esters were accountable for the green synthesis of the gold and silver nanoparticles (AuNPs and AgNPs). The size of smallest particle of AuNPs was confirmed to be 20 nm and that of largest particle to be 50 nm. However, the size range of AgNPs was confirmed to be 20 nm – 80 nm. The silver nanoparticles were found to be spherical and cubic in nature.

The efficiency of the plant extracts against microbial strains was increased by the nanoparticles. The silver nanoparticles (AgNPs) were found more effective in controlling the growth of bacterial and fungal strains when compared to gold nanoparticles (AuNPs). More significant results were recorded by roots of *Viola pilosa* and leaves of *Skimmia laureola* against bacterial strains. Similarly, shoots extracts of *Viola pilosa* and stem extracts of *Skimmia laureola* were found more efficient in antifungal activities. The antioxidant potential of gold and silver nanoparticles (AuNPs, AgNPs) from the roots and shoots of *Viola* as well as from the leaves and stem of *Skimmia* proved that all the tested concentrations of silver and gold nanoparticle from both plants were active in scavenging free radicals possessing significant antioxidant potential when compared to the control. Similarly, roots extracts of *Viola pilosa* were by far the most potent in scavenging free radicals amongst all the other solvent extracts and showed much significant anioxidant activities. Different parts of both plants also presented significant level of phytotoxic and insecticidal activities pointing towards the potential possibility of these plants to be used as pesticides and herbicides. Phytochemical study of the test plants
discovered that they were abundant in tannins, carbohydrates, sterols, proteins and lipids,

Keeping the above conclusions in view, the following commendations can be safely deduced,

- Biogenic AuNPs and AgNPs from both the test plant species can be effectively used as powerful carriers of drug for different antibiotics.
- These NPs from different extracts could be further linked and stabilized with different polymers for functioning with DNA, RNA or some other compounds, that can be successfully applied in biomedical sciences.
- To the best of our understanding, we are the very first to study the insecticidal and phytotoxic potentials and optimize the protocol of biogenic synthesis of gold and silver NPs from methanolic crude and aqueous extracts at such a lower concentration of metal salts.
LITERATURE CITED


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APPENDICES

APPENDIX – I

Represents the extract (A) Formation of AuNPs (B). The color change from yellow to purple.
APPENDIX – II

Fractionation of the crude methanolic extract.
APPENDIX – III

1. Antifungal activity of *Skimmia* leaves crude against *C. albicans*.

2. Antifungal activity of *Skimmia* leaves butanol against *A. niger*.
3. Antibacterial activity of *Skimmia* leaves crude against *K. pneumoniae*.

4. Antibacterial activity of *Skimmia* stem crude nanoparticles against *S. aureus*. 
APPENDIX – IV

Antioxidant activity of plant extracts.
APPENDIX – V

Phytotoxic activity of the plant extracts.
APPENDIX – V

Insecticidal activity of the plant extracts.
APPENDIX – VI

Qualitative analyses of the plant extracts.